# Characterization of the Reproductive Toxicity of Depleted Uranium

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The purpose of the proposed work is to determine the reproductive and developmental toxicity of depleted uranium (DU) ore in Sprague-Dawly rats. DU-tipped munitions have become common-place on the modern battlefield and recent experience has shown that minor injuries associated with DU munitions are becoming more common. Four hundred ninety-eight adult (P1) SD rats were implanted with up to 20, 1x2 mm DU pellets and mated at 30 and 120 days post-implantation. Preliminary findings indicate no adverse effect associated with DU implantation on P1 reproductive success, or F1 and F2 offspring survival and development.

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Introduction:

This is the final (Year 3) annual report for DAMD17-02-IA-0003 Characterization of the Reproductive Toxicity of Depleted Uranium. This was a 3-year project that focused on developing data to fill existing gaps in knowledge on the reproductive and developmental effects of implanted depleted uranium (DU). Beginning in July 2003, rats were implanted with up to 20 1 x 2 mm DU pellets and mated to produce an F1 generation. F1 survival and development was monitored through post natal day (PND) 90. Select F1 pups were cross-mated with pups from different litters of the same treatment group to produce an F2 generation. F2 survival and development was monitored through post natal day (PND) 90. Select P1, F1, and F2 rats underwent neurobehavioral and immune function testing. All animal studies were completed by February 2005.

Body:

## STUDY DESIGN

The reproductive toxicity of implanted depleted uranium pellets was assessed in a 2-generation reproductive toxicity format following OECD test guidelines (OECD 1983, 1999). Adult rats (e.g., P1 generation) were implanted with up to 20, 1 x 2 mm DU pellets and mated at 30 and 120 days post-implantation with a mate implanted with the same number of pellets. Offspring produced after mating at 30 days postimplantation (F1a) were monitored through post natal day (PND) 50 and in some cases up to PND 120. Offspring produced after mating at 120 days postimplantation (F1b) were monitored through PND70 and then mated with an F1b member derived from the same parental dose group and from a different litter. Offspring produced after F1b mating (e.g., F2 offspring) were then monitored through PND 90. A schematic of the study design is shown in Figure 1. Parameters measured in the study for each of the generations are listed in Table 1.

The surface area of 4, 8, 12, and 20 1 x 2 mm DU pellets approximates to 31, 63, 94, and 157 $mm^2$ respectively, and is 0.1%, 0.3%, 0.4%, and 0.6% respectively, of the estimated body surface area of an adult rat (SA=0.025 $m^2$). Twelve, 1 x 2 mm DU pellets is equivalent to one 30 mm APFSDS-T DU solid projectile (≈ 425 mg DU, 28 cm (11 in) in length). Twenty 1 x 2 mm DU pellets (760 mg of DU) in a 250 g rat is equal to approximately 0.22 kg (0.5 lb) of DU in a 70 kg (154 lb) person.

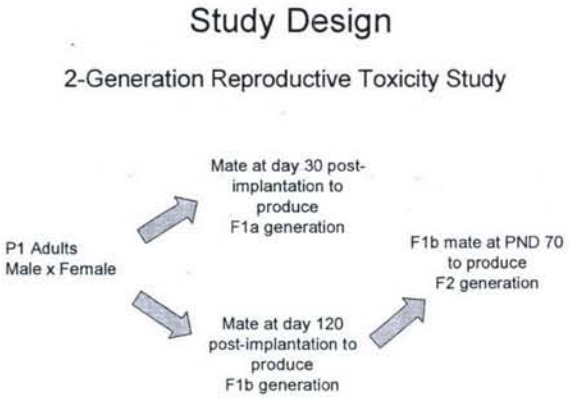



**Figure 1: 2-generation reproductive toxicity study**

## METAL PELLETS

Cylindrical depleted uranium (DU) alloy (99.2% DU, 0.8% titanium) pellets, 1 x 2 mm diameter, were obtained from the United States Department of Energy, Y-12 National Security Complex (BWXT Y-12), Oak Ridge, TN., under DOE Project Number 2348-S535-A1. The 1 x 2 mm pellets used in this study were milled from a single 120 mm APFSDS projectile. As a quality control measure, 2 pellets were randomly selected from every 50 pellets milled and their 1 x 2 mm dimensions were verified by Y-12 scientists. Cylindrical Ta steel (e.g., "surgical steel") pellets, 1 x 2 mm, were manufactured by and purchased from Alfa Aesar (Ward Hill, MA).

## SURGICAL IMPLANTATIONS

Male and female Sprague-Dawley rats (4 weeks of age) were obtained from Charles River Laboratories (Raleigh, NC) and housed in an AAALAC accredited environmental control animal facility at Wright-Patterson AFB (Dayton, OH). At 8 weeks of age, the animals were surgically implanted with DU or Ta pellets using the methods previously described by Pellmar et al. (1999) except isoflurane vapor was administered as a general anesthetic using an open circuit system with a scavenger/recapture system (IMPAC[6], VetEquip, Inc., Pleasanton, CA).

Males were implanted with 0 (e.g., sham surgery controls), 12 or 20 DU alloy pellets or 12 or 20 Ta steel pellets. Females used in the mating tests were implanted with 0, 12, or 20 Ta steel pellets or 4, 8, 12, or 20 DU pellets. Ta steel pellets served as inert controls to account for possible adverse effects on well-being and reproduction associated with implantation of solid, 1 x 2 mm objects in the rat gastrochnemius. Ta pellets have been used previously in DU implantation studies as inert foreign-body controls (e.g., Benson 1998; Pellmar et al. 1999; Hahn et al. 2002). Two, four, six or ten pellets were implanted in each gastrochnemius muscle resulting in a total of four, eight, 12 or 20 pellets implanted per rat.

The physical well-being of each animal was assessed once daily for signs of debilitating toxicity and disease listed in OECD Guideline No. 19 by AALAS-accredited veterinary technicians until the end of the P1 adult reproductive segment of the study at post-implantation day 15 (OECD 2000).

## ANIMAL MANIPULATIONS

*Measurement of urinary uranium of implanted P1 animals*

Ten male and 10 female P1 animals from each of the 13 treatment groups listed in Table 2 were placed in Nalgene metabolism chambers for 24 hours at 27 and 117 days post-implantation. Urine was collected and analyzed for uranium content. Animals were allowed access to food and water *ad libitum* before being placed into metabolism chambers and throughout the 24-hour collection phase. Urine samples were prepared for analysis and analyzed for uranium content by ICP-MS using EPA Method 6020. The Limit of Detection (LOD) for uranium in urine was 10 µg/L.

*Mating of P1 generation and monitoring of pregnancy and offspring*

Surgically-implanted adults (P1 generation) were mated at 30 days post surgical implantation as summarized in Table 2. P1 males were mated with P1 females within the same treatment group. Males and females within the same treatment group were paired on a random basis for mating.

Male/female pairs were placed in open metal grid mating cages for 7 days. The bottoms of the mating cages were checked twice daily by two trained technicians for evidence of mating (e.g., seminal plugs). Appearance of a seminal plug was considered evidence of a mating success and the average time to insemination was calculated by subtracting the day of the appearance of a seminal plug from the date that the male and female were placed together in a mating cage. If no evidence of mating was found for the mated pair by day 8, the male was returned to its home cage and the mating recorded as a "failure". The female was then paired for 7 days with a male from the same treatment group that had a first-time mating success. The cage was checked twice daily by two trained technicians for seminal plugs and the average time to insemination calculated If no evidence of mating was found for the mated pair, the male was returned to its home cage on mating day 8 ($2^{nd}$ mating attempt) and the mating was recorded as a mating "failure".

In the interest of achieving adequate statistical power for detecting decrements in mating success, it was decided to pool mating success data for male rats implanted with 12 or 20 DU or Ta steel pellets *regardless* of the implantation status of their female mates. Male reproductive success data were pooled for the following groups: Groups 3-6, Groups 2, 7-9, Groups 10 and 12, and Groups 11 and 13. This was justified since female mating success did not differ by increasing numbers of implanted DU or Ta steel pellets. Furthermore, female P1 neurobehavior, gestation weight gain, and gestation length did not differ by the number of DU or Ta steel pellets implanted supporting the conclusion that there was no effect of increasing number of implanted pellets on female mating success.

All P1 females were monitored for significant weight gain on gestation day (GD) 5, 10, 15, and 20, including P1 females that did not produce evidence of a vaginal plug. Significant weight gain without evidence of a vaginal plug was considered to be a pregnancy and the initial score of "mating failure" was changed to "mating success". Significant weight gain through GD 10, 15, or 20 without delivery of pups between GD 19-25 was considered evidence of whole litter resorption.

Beginning on GD 19, P1 dams were monitored frequently for evidence of birth. Upon giving birth, the number of pups born in each litter was recorded as soon as possible. For all litters, the number of males and females and a collective litter weight were recorded on the first full day after birth. The general physical condition of the litter, number of pups per litter, and number of malformations per litter, was assessed at least twice daily from PND1-4 and at least daily thereafter. No attempts were made to augment or supplement maternal care at any time during the study. Dead pups were examined for gross or obvious defects, and if found within 2-3 hours of death, were placed in 10% formalin for potential future analysis. On postnatal day (PND) 4, if necessary, litters were culled to 8 pups in close to equal male:female ratio as possible. In cases where the litter was culled to 8 pups, up to four carcasses (2 male and 2 female) were frozen at 4°C and sent to NEL Laboratories for whole body uranium concentration analysis. Whole body uranium concentrations were measured by ICP-MS using EPA Method 6020. The LOD of detection of uranium in PND 4 whole body homogenates ranged from 450-600 µg/kg and was inversely dependent on the mass of the PND 4 pup. The LOD of uranium in PND 20 whole body homogenates ranged from 420 – 550 µg/kg.

Surgically-implanted adults (P1 generation) were mated at 120 days post surgical implantation as summarized in Table 2 and using the methods described previously. P1 males were mated with P1 females within the same treatment group. Males and females within the same treatment group were paired on a random basis for mating. P1 males were mated with a different female within the same treatment group at the 2$^{nd}$ mating cycle beginning post-implantation day 120.

Select P1 animals underwent neurobehavioral assessments beginning 150 days post-implantation. Animals were evaluated in the Spontaneous Locomotor Activity (SLA) Test, Acoustic Startle/Pre-pulse Inhibition (AS/PPI) Test, the Morris Watermaze, and the Conspecific Social Approach Test (see Neurobehavioral Assessments).

All surviving P1 animals were euthanized and underwent necropsy by 200 days post-implantation. Necropsy procedures and post-necropsy tests are described in the Methods section entitled "Necropsy".

**F1a GENERATION**

P1 females were allowed to nurse and care for the culled F1a litters through PND 20. All F1a offspring were weighed on PND20 and the P1 mother was returned to her home cage. Two male and 2 female F1a PND20 pups were randomly selected from the litter and anesthetized until unresponsive by $CO_2$ overdose. The animals were then euthanized by exsangination via the vena cava. Of the 4 euthanized animals, 1 male and one female pup was preserved in 10% formalin for potential future pathological evaluation; the other male and female pup was sent to NEL Laboratories for whole body uranium concentration analysis.

One male and one female from each F1a litter were single-housed and their survival and body weight gain was monitored until PND90. Select F1a animals underwent immune function testing beginning PND56. Select F1a animals underwent neurobehavioral testing beginning PND17 through PND63. Surviving F1b animals were euthanized and underwent necropsy by PND90. Necropsy procedures and post-necropsy tests are described in the Methods section entitled "Necropsy".

**F1b GENERATION**

P1 females were allowed to nurse and care for the culled F1b litters through PND 20. All F1b offspring were weighed on PND20 and the P1 mother was returned to her home cage. Two male and 2 female F1b PND20 pups were randomly selected from the litter and anesthetized until unresponsive by $CO_2$ overdose. The animals were then euthanized by exsangination via the vena cava. Of the 4 euthanized animals, 1 male and one female pup was preserved in 10% formalin for potential future pathological evaluation; the other male and female pup were sent to NEL Laboratories for whole body uranium concentration analysis.

Two males and two females from each F1b litter were single-housed and their survival and body weight gain was monitored until PND70. At PND70, F1b animals were mated to produce the F2 generation. F1b males and F1b females were mated by parental treatment group in that the P1 parent of both F1b males and females were from the same treatment group listed in Table 2. Mated F1b males and F1b females were derived from different litters to ensure that the F1b mated pairs were not related parentally.

F1b male/female pairs were placed in open metal grid mating cages for 7 days. The bottoms of the mating cages were checked twice daily by two trained technicians for evidence of mating (e.g., seminal plugs). Appearance of a seminal plug was considered evidence of a mating success and recorded for the male. The average time to insemination was calculated by subtracting the day of the appearance of a seminal plug from the date that the male and female were placed together in a mating cage. If no evidence of mating was found for the mated pair by day 8, the male was returned to its home cage and the mating recorded as a "failure". The female was then paired for 7 days with a male from the same treatment group that had a first-time mating success. The cage was checked twice daily by two trained technicians for seminal plugs and the average time to insemination calculated If no evidence of mating was found for the mated pair, the male was returned to its home cage on mating day 8 ($2^{nd}$ mating attempt) and the mating was recorded as a mating "failure".

All F1b females were monitored for significant weight gain on gestation day (GD) 5, 10, 15, and 20, including F1b females that did not produce evidence of a vaginal plug. Significant weight gain without evidence of a vaginal plug was considered to be a pregnancy and the initial score of "mating failure" was changed to "mating success". Significant weight gain through GD 10, 15, or 20 without delivery of pups between GD 19-25 was considered evidence of whole litter resorption.

F1b dams were monitored twice daily for evidence of F2 birth beginning on GD 19. The number of F2 pups born in each litter was recorded as soon as possible after birth. For all F2 litters, the number of males and females and a collective litter weight were recorded on the first full day after birth. The general physical condition of the litter, number of pups per litter, and number of malformations per litter, was assessed at least twice daily from PND1-4 and at least daily thereafter. No attempts were made to augment or supplement maternal care at any time during the study. Dead F2 pups were examined for gross or obvious defects, and if found within 2-3 hours of death, were placed in 10% formalin for potential future analysis. On postnatal day (PND) 4, if necessary, F2 litters were culled to 8 pups in close to equal male:female ratio as possible. In cases where the F2 litter was culled to 8 pups, up to four carcasses (2 male and 2 female) were frozen at 4°C and sent to NEL Laboratories for whole body uranium concentration analysis.

F1b females were allowed to nurse and care for the culled F2 litters through PND 20. All F2 offspring were weighed on PND20 and the F1b mother was returned to her home cage. Two F2 male and 2 female PND20 pups were randomly selected from the litter and anesthetized until unresponsive by $CO_2$ overdose. The animals were then euthanized by exsangination via the vena cava. Of the 4 euthanized animals, 1 male and one female pup was preserved in 10% formalin for potential future pathological evaluation; the other male and female pup were sent to NEL Laboratories for whole body uranium concentration analysis.

F1b male and female survival and body weight gain were monitored until PND 200. Select F1b animals underwent immune function testing beginning PND 56. Select F1b animals underwent neurobehavioral testing beginning PND17 through PND63. Surviving F1b animals were euthanized and underwent necropsy by PND 200. Necropsy procedures and post-necropsy tests are described in the Methods section entitled "Necropsy".

## F2 GENERATION

F2 male and female survival and body weight gain were monitored until PND 90. Select F2 animals underwent immune function testing beginning PND 56. Surviving F2 animals were euthanized and underwent necropsy by PND 90. Necropsy procedures and post-necropsy tests are described in the Methods section entitled "Necropsy".

## NECROPSY

On the day of the necropsy, male and female animals were anesthetized until unresponsive by $CO_2$ overdose. Female animals were euthanized by exsangination via the vena cava. Male animals were euthanized by rapid decapitation and both distal cauda epididymis were rapidly removed. One cauda was used for assessing sperm motility, and the other cauda was used for determining caudal sperm concentration. Blood was

collected, processed and prepared for morphology and clinical chemistry analysis following standard laboratory procedures (e.g., Stiene-Martin and Lotspeich-Steingelt, 1992).

Necropsy was performed by making a ventral midline incision extending from the level of the mandible to the pelvis. The skin was then reflected laterally to facilitate examination of the thoracic and abdominal organs. The thoracic cavity was exposed by removing the ribcage along the cartilaginous junction. The top portion of the skull was removed to facilitate examination of the brain. Pellet implantation sites (e.g., gastocnemius) were examined for evidence of tumors, infections, or other abnormalities.

*Tissue Histopathology*

Whole formalin fixed tissues were submitted to Colonel Jeffery Eggers (VC, USA), FRL-HEDV, Brooks City Base, TX., for histopathological analysis. The following tissues were prepared for histological evaluation: lung, spleen, thymus, kidney, liver, skeletal muscle, uterus, ovaries, testes, bone marrow. Requested tissues were identified, embedded in paraffin and trimmed, sectioned, and stained with standard H&E stains. In most females, the uterus was submitted without the ovaries still attached. When the ovaries could not be located (all but 2 females), a cross section of the uterus was processed for evaluation. Each sample of skeletal muscle was bread-loafed in an attempt to identify implant sites. If implants were identified grossly when trimming, these areas were processed for histologic evaluation.

*Measurement of sperm motility and concentration*

The sperm motility was measured following methods established by Slott et al. (1994). Each excised cauda epididymis was clamped longitudinally with hemostats and the distal end punctured 3 times with an 18-gauge needle. The cauda was then placed in a petri dish with 500 ml M199 solution (buffered Earles salts, sodium bicarbonate, L-glutamine, and HEPES buffer) Invitrogen Life Technologies (Carlsbad, CA) and incubated at 37° C for 5 minutes to allow sperm swim-out. A 100 µL portion of the sperm sample was diluted with 200 µl of M199/BSA solution in a pre-warmed microcentrifuge tube. A 15 µl portion of the diluted sample was transferred to a Hamilton Thorne sperm analysis chamber (Beverly, MA). Percent motile sperm, percent of progressively motile sperm, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), and amplitude of lateral sperm head displacement (ALH) were measured using a Hamilton Thorne IVOS-12 sperm analyzer (Beverly, MA). The "standard rat" was selected in the IVOS-12 subroutines for sperm motility and sperm motion analysis. Motility and motion parameters were measured from sperm present in a minimum of six standard viewing fields. A minimum of 200 sperm were analyzed per animal. Percent progressive sperm is the ratio of the number of motile sperms with both a path velocity (VAP) > $V_0$, and straightness (STR) > $S_0$, to the total number of sperm (Kato et al. 2001). Percent progressive sperm and the sperm motion parameters VCL, VSL, VAP, and ALH

have all shown to be sensitive measures for detecting adverse effects on sperm motion (Kato et al. 2001).

Sperm concentrations were measured with the Hamilton Thorne IVOS-12 sperm analyzer using the HTM-IDENT option as described by Strader et al. (1996). Epididymides were removed at necropsy and frozen at -20°C until analyzed. Frozen epididymides were thawed at room temperature for 2 hours. Each epididymis was weighed and homogenized in 15 mL of deionized $H_2O$. One hundred microliters of the diluted homogenate and 100 µL of deionized $H_2O$ were placed into a Hamilton Thorne IDENT stain reaction vile containing a pellet of dehydrated *bis* benzimide trihydrochloride. The mixture was vortexed and allowed to incubate at room temperature for 5 minutes. The tube was occasionally re-vortexed for 10-20 seconds during the incubation period to promote staining of the sperm DNA with *bis* benzimide trihydrochloride. Immediately following the incubation period, 8 µL of the sperm solution was transferred to a 20 µm Cell-VU chamber (Fertility Technologies, Natick, MA). The standard setting in the HTM-IDENT program for rat cauda sperm (Set D) was used for sperm count analysis. The settings used were as follows: Frame Rate: 60Hz; Frames Acquired: 12; Minimum Contrast: 53; Minimum Cell Size: 2; Threshold Straightness: 80; Medium VAP Cut-off: 25; Low VAP Cut-off: 5; Low VSL Cut-off: 30; Static Size Limits: 0.35-2.00; Static Intensity Limits: 0.83-2.00; Static Elongation Limits: 15-84; and Eield Selection: Automatic. The 10x UV objective was used for analysis; the magnification setting obtained was 1.95. Ten fields were analyzed per sample. Results for each of the 10 fields were summed and an average sample concentration was calculated for each sample.

An additional 20 male Sprague-Dawley rats (6 weeks of age) were obtained from Charles River, (Raleigh, NC) and assigned to the α – chlorohydrin (ACH) positive control group. A single oral gavage dose of ACH (100 mg/kg) has previously been shown to significantly reduce rat sperm motility within hours of exposure (Vetter et al. 1998, Wier and Rumberger 1995). At 8 weeks of age, each of the 20 positive control males were given a single oral dose of α – chlorohydrin (CAS: 96-24-2) at 100 mg/kg dissolved in deionized water. The animals were euthanized by rapid decapitation 3 hours post-dose and sperm motility was measured.

*Serum chemistries*

Serum chemistries were measured using a VetTest® Snap Reader (IDEXX Laboratories, Inc., Westbrooke, ME). A 100 µL sample of serum from each animal was analyzed for total protein (g/dl), alkaline phosphatase (ALKP) activity, alanine aminotransferse (ALT) activity, urea/BUN concentration (mg/dl), creatinine (CREA) concentration (mg/dl), glucose concentration (mg/dl), phosphatase (PHOS) concentration (mg/dl), and total bilirubin (TBIL) concentration (mg/dl). Serum chemistries were not measured for P1 rats implanted with 20 Ta steel pellets.

*Hematology*

Blood samples were processed for clinical chemistry analysis following standard laboratory procedures (Stiene-Martin and Lotspeich-Steingelt, 1992). Blood cell counts were performed on 50 µl whole blood samples per animal using a Coulter ACT Diff 2 (Beckman Coulter, Fullerton, CA). Parameters measured were number of white blood cell (WBC) per µL, % lymphocytes, % monocytes, number of granulocytes per µL, number of lymphocytes, monocytes, granulocytes, and red blood cells (RBC) per µL, grams of hemoglobin (HgB) per dl, % hematocrit, mean corpuscle volume (MCV), mean corpuscular hemoglobin (MCH) per pg, mean corpuscular hemoglobin concentration (MCHC) per dl, red blood cell distribution width % (RDW), red blood cells with pits (PIT) per µL, and mean platelet volume (MPV).

*Rib cage abnormalities*

Rib cages from PND 20 pups were removed at necropsy using techniques described by M.S. Christian in *Principles and Methods of Toxicology-Fourth Edition* (edited by A. Wallace Hayes). The rib cages were placed in 50 mL centrifuge tubes and 40 mL of 1% KOH + 10mg/L of Alizarin Red S were added. The ribcages were then placed on a rocker set at slow speed and the ribs were incubated at room temperature for 48 hours. After 48 hours, the solution was decanted and the ribs were rinsed with tap water. Forty milliliters of 50% glycerol + 35% ethanol was added to each tube containing a ribcage. The tubes were then placed at 4°C for storage.

Rib cages were evaluated for abnormalities following established guidelines (M.S. Christian in *Principles and Methods of Toxicology-Fourth Edition*, edited by A. Wallace Hayes, 2001, Philadelphia, PA, pp. 1301-1381). Rib cages were evaluated independently by a trained veterinary pathologist (MAJ Mary Cooper, Brooks City Base, TX) and two study technicians with experience in animal toxicology studies. All persons involved with evaluating the rib cages were blinded to the pup's treatment status. Ribs present in each rib cage were counted and evaluated for abnormalities such as: waviness, branching, fusion, misalignment, or thickened areas of ossification as depicted in photographs on pages 1359-1361 of *Principles and Methods of Toxicology-Fourth Edition* (edited by A. Wallace Hayes). The number of ribs present and abnormalities (if any) were recorded and tabulated.

**NEUROBEHAVIORAL ASSESSMENTS (Dr. Marni Bekkedal, State of Wisconsin, Bureau of Environmental and Occupational Health, Department of Health and Family Services, Madison, WI)**

Neurobehavioral assessments were conducted to gauge the effect, if any, of surgical implantation with DU pellets on P1 neurobehavior, P1 and F1b maternal retrieval of separated litter mates, and early (e.g., PND 5-7) and late (e.g., PND 17-150) neurobehavioral development of F1a and F1b pups.

*F1a and F1b neurobehavioral testing*

To screen for early markers of neurodevelopmental effects in the pups, four tests were conducted: righting reflex, ultrasonic vocalizations, age of eye opening, and juvenile social interaction. Selection and execution of these early tests for neurobehavioral integrity closely followed those used in a previous study based on the comprehensive Navy Neurobehavioral Toxicology Assessment Battery (NTAB) where pups were evaluated for indicators of neurodevelopmental abnormalities related to pre- and neonatal exposure to a neurotoxic convulsant (Bekkedal *et al.* 1998; Bekkedal *et al.* 1999). The NTAB includes over 30 tests to evaluate neurobehavioral capacities throughout the lifespan (Rossi, III *et al.* 2000). Those used in the current research were selected to specifically address developmental markers for basic proprioceptive coordination, social bonding (Hofer *et al.* 1989; Winslow and Insel 1991), social interaction (Panksepp *et al.* 1984), and the classic neurodevelopmental milestone of age of eye opening.

a. Maternal Retrieval: On PND 2, dams were tested for retrieving their pups and returning them to the nest. Each dam was briefly removed from the home cage and placed in a holding cage while 3 pups were randomly selected, removed from the nesting area and placed at the opposite end of the home cage. The dam was replaced in the home cage at the nest area with the remaining pups, and timing was started. The latency for the dam to retrieve all 3 pups and return them to the nest was measured. If the dam did not retrieve all 3 pups within 5 min, then a score of "timed out" was recorded for that litter.

b. Righting Reflex: Pups were tested for the righting reflex on PND 4. Pups were individually placed on a platform in the supine position by gently using the forefinger running the length of the body and applying light pressure to the ventral surface. A timer started immediately upon release of pressure from the forefinger and continued until the pup had rolled over such that all 4 paws were placed flat on the platform. The test was immediately repeated twice more for a total of 3 consecutive tests per pup. Testing was conducted for all pups in the litter. If a pup failed to complete the task within 60 sec, "timed out" was recorded and the pup was picked up from the platform, and then replaced in the supine position, and remaining tests continued.

c. Ultrasonic Separation Distress Vocalizations: On PND 7, pups were tested for emission of distress vocalizations upon separation from the dam and littermates. Pups were individually placed in the center of a glass bell jar (9 cm diameter X 12 cm height) lined with 1-2 cm of bedding from the home cage. The jar was located on a platform in a room lit only with dim red lighting. A Peterson Ultrasound Detector D 240 tuned to 40 kHz was hung over the center of the jar such that the microphone was 15 cm from the top of the jar. Sounds were digitally recorded on a Tascam DA_20 MKII DAT recorder on 44.1k recorder mode for 60 sec beginning immediately after the pup was placed in the jar. Afterwards the pup was placed back with the dam and littermates and the next pup was tested until all of the litter was tested. Vocalizations were manually scored from the tape recordings, and the number of vocalizations for each animal was recorded.

d. Age of Eye Opening: All pups in each litter were checked daily for eye opening. The ages for the first male and first female in each litter were recorded, as was the litter's age when the eyes of all animals within the litter had opened.

e. Juvenile Play: Littermate pairs of rats were evaluated for early social interaction as indicated by juvenile play behavior while in the age range of PND 17 – PND 32. Test sessions were repeated once a day for 4 consecutive days. Descendents from P1 Group 1, Group 2, Group 5, Group 6, Group 10, and Group 11 were tested.

Pups were paired to match for litter, gender, and body weight whenever possible. Any incidents where there was a greater than 10% difference in body weight were noted. Pups were housed separately throughout the play test days. They were individually weighed, and one member of each pair was marked on its dorsal surface with non-toxic, odorless black ink for identification purposes during the play bout. Pairs were placed inside a Plexiglas box lined with 2-3cm of bedding and lit with a dim red light. The box was placed in a sound attenuating chamber in a dark room, and the activity of the pair was videotaped for 5 minutes. At the end of the session, each pup was returned to its individual home cage.

The play session was manually scored from the videotape. The frequency of dorsal contacts and pins, and the average duration of a pin were recorded for each pup in the pair. A dorsal contact was scored when the animal placed its front paws on the dorsal surface of the play partner. A pin was scored when the partner was rolled onto the dorsal surface and held in place for at least 1 second by the animal. A total duration for pins during the session was scored, and used to calculate the average duration per pin.

f. Spontaneous Locomotor Activity (SLA) Test (PND 32): The SLA assesses gross locomotor movements, stereotypy, exploratory behaviors, and emotionality. On PND 32, pups were placed in automated Opto-Varimex Animal Activity Monitors in a testing area lit with low illuminating red light (25-40 W). Activity was monitored on 17" x 17" Plexiglas open fields using infrared photocells aligned 1 inch apart. The photocells were designed to detect both horizontal and vertical movements, as well as differentiate small (stereotypic) movements from large movements. Each animal was placed in the center of the open field and monitored for up to 90 minutes. Open field activity grids were washed down after each use with a solution of 10% ethanol to remove olfactory cues.

g. Acoustic Startle/Pre-pulse Inhibition (AS/PPI) Test (PND 45-47): The AS test is a measure of the integrity of the auditory reflex centers in the mammalian brainstem, whereas the PPI test is a measure of the inhibitory controls over the reflex arc normally provided by higher level control centers. Pups were placed in steel tubes so their heads were oriented in the direction of an audio speaker. The tube was positioned on a movement-sensitive platform within a standard sound and light attenuated chamber. Background white noise was present throughout a testing session.

The acoustic startle test began with a 60 second adaptation period, followed by 8 discrete blocks of 10 trials. All of the trials within a block consisted of 1 of 3 conditions: pre-

pulse tone only, startle tone only, or pre-pulse + startle tones. The pre-pulse tone was 74dB, which generates a mild, if any startle movement in the animal. The startle tone was 105dB and reliably elicited a reflexive startle or "jerk." The startle response becomes attenuated when the pre-pulse tone immediately precedes the startle tone (pre-pulse + startle). Startle movements were detected by the platform. Each testing session was 25 minutes long divided into 8 testing blocks (Table 3) and included a 60 second adaptation period before block 1, and between all remaining blocks. Within a block, the tones were separated by approximately 10 seconds. Testing was started on PND 45 and continued through PND 47.

h. Morris Watermaze (PND 59-63) The MWM is a test used to evaluate visuospatial learning, spatial navigation, and long term and short term spatial memory. A rat is placed in a 4' diameter cylindrical metal tank (2' high) filled nearly to the top with water (maintained at 22-25°C) containing non-toxic, white tempera paint (1%) such that the water is opaque. Attached to the floor of the tank, but submerged 1" below the surface of the water, is a round escape platform large enough for the animal to stand on. The animal is placed in the water at a location distal from the escape platform. The rat is allowed to swim until it reaches the escape platform. Once it finds the platform and climbs onto it, the rat is removed from the tank, dried with a towel, and placed in its home cage until the next trial. Rats are trained for five trials per day, separated by 10-20-min each, until they consistently swim to the platform in less than 15 seconds. If a rat does not locate and stand on the platform within 600 seconds, it is removed from the water, dried with a towel, and returned to its home cage. Rats are immediately removed from the water if the nares of the nose become submerged for 3 seconds. The following measures are recorded electronically using a Video-Max video tracking system:

(i) Latency from starting point to platform (escape latency).
(ii) Time in the appropriate maze quadrant.
(iii) Swimming distance & average swimming speed.
(iv) Time circling.
(v) Time "wall swimming".
(vi) Immobility (floating) time.

Once the animal achieves the task criterion performance level of 5 consecutive sessions of locating the platform in less than 15 seconds, the visual cues will be altered by changing the alignment of the tank with the room by rotating it 90 deg and the rat will be retested to assess relearning skills. All procedures and completion criteria will be the same as in the initial training/testing.

*P1 neurobehavioral testing*

Select P1 animals underwent neurobehavioral assessments beginning 150 days post-implantation. Animals were evaluated in the Spontaneous Locomotor Activity (SLA) Test, Acoustic Startle/Pre-pulse Inhibition (AS/PPI) Test, the Morris Watermaze, and the Conspecific Social Approach Test.

Conspecific Social Approach Test: The conspecific approach test is used to assess social interaction in adult rats (Panksepp, et al. 1997). Rat social interaction is assessed by measuring the frequency of exploration of social "holes" versus non-social holes in the testing apparatus. One animal is placed in each compartment of the test apparatus (2 plexiglass boxes or chambers with "exploration holes" cut into each end wall). The holes cut into the end walls allow animals to poke their heads through to explore but do not allow the animal to exit the test box. The test was started by aligning two test boxes containing animals end-to-end so that the end holes faced each other (social holes) and the non-social holes at the opposite ends of the boxes faced out. Photosensors recorded the frequency of test animals exploring social and non-social holes. Tests were conducted in a room dimly lit with a 25-40 W red light. Exploration frequency was recorded for a minimum of 30 minutes and a maximum of 90 minutes. Exploration frequency was recorded and tabulated via computer.

**IMMUNE FUNCTION TESTS (Dr. Keith Grasman, Wright State University, Dayton, Ohio)**

The immune endpoints evaluated during the course of this study included: thymus mass relative to body mass, thymocyte viability, thymocyte apoptosis determine by flow cytometry, T-cell induced delayed type hypersensitivity (DTH), and an ELISA assay to measure IgM mediated antibody response. The thymus based assays and the DTH test evaluated T-cell development and function respectively. The ELISA evaluated B-cell function.

Immune function testing was performed on P1 adults and young adult stage F1a, F1b, and F2 offspring (starting on PND 56, or 8 weeks of age). For P1 males, immune function was assessed for animals implanted with 12 Ta steel pellets versus animals implanted with 12 DU pellets. For P1 females, immune function was assessed for animals implanted with 12 Ta steel pellets versus animals implanted with 4, 8, or 12 DU pellets. For F1a, F1b, and F2 animals, immune function was assessed for males and females from each of the 13 P1 treatment groups.

Immune function of 8 week-old positive control animals was intentionally suppressed by treatment with cyclophosphamide (CPA). CPA suppresses T, B, and NK cell function, and induces thymic atrophy and thymocyte apoptosis (Talcott *et al.* 1985; Exon *et al.* 1986; O'Reilly and Exon 1986; Wang and Cai 1999). At approximately 56, 70, and 76 days of age, positive control animals were injected IP with CPA. These injections occurred 48 hours before primary SRBC (sheep red blood cell) injection, secondary SRBC injection, and Delayed-Type Hypersensitivity (DTH) test injections.

a. Delayed-Type Hypersensitivity (DTH) Test

On PND 20, 1 male and 1 female pup were randomly selected from each litter from each of the 9 P1 treatment groups. Pups of the same gender from parents of the same treatment group were single or double housed until approximately 56 days of age. Upon

reaching test age, F1 pups under isoflurane anesthesia were injected in the lateral tail vein with 0.5ml of 2 x $10^8$ SRBCs in saline. Two weeks following primary SRBC immunization, the animals received an identical secondary immunization. Six days after the second SRBC immunization, the thickness of both hind footpads was measured using pressure-sensitive calipers (Dyer, Lancaster PA). The footpad of one hind foot was injected intradermally with 1x$10^8$ SRBCs contained in a total volume of 0.05 ml of saline and footpad of the other hindfoot was injected with 0.05 ml of saline to serve as a negative control. The thickness of both footpads was then measured 24±3 hours after injection. Thickness was measured using a footpad stimulation index for each animal tested was then calculated by subtracting the increase in thickness of saline-only injected footpad from the increase in thickness of the SRBC-injected footpad. CPA-treated animals underwent identical testing procedures and their footpad stimulation indices were compared with pups derived from mothers from Groups 1-13.

b. Assessment of spleen and thymus

Upon completion of DTH testing, pups and CPA-treated animals were euthanized by $CO_2$ overdose and exsanguination via the vena cava. Spleen and thymus were removed and weighed at necropsy. Average absolute spleen and thymus weights were calculated and compared between CPA-treated animals versus pups derived from mothers from Groups 1-13.

For each animal, whole thymus was homogenized in phosphate buffered saline (PBS) using a Kontes tissue grinder. Trypan blue was added to a diluted aliquot of the homogenates and a count of live versus dead thymocytes was done using a Neubauer hemacytometer (Hausser Scientific, USA) under 400x magnification. Counts of live versus dead cells in the stained homogenate were performed. Average total number of cells per thymus, number of live cells per thymus, number of dead cells per thymus, and percent viable cells were then compared for CPA-treated animals versus pups derived from mothers from Groups 1-13.

c. Analysis of thymus cell apoptosis

Three million cells were fixed for later cell cycle analysis using flow cytometry similar to Nicolleti et al. 1999. Briefly, 3 million cells were pelleted and supernatant was removed. The pellet was re-suspended using 600µl PBS, and transferred to a 5ml capped tube. Then, 1.4ml of -20°C ethanol was slowly added while the tube was vortexed gently. One day to one week after fixation, samples were pelleted and supernatant removed. The pellet was resuspended in 0.5ml PBS then treated with 0.5ml RNase. Then, 1ml propidium iodide was added and the samples were placed in the dark in a 37°C incubator for half an hour. Samples were analyzed using a Becton Dickenson FACScan benchtop flow cytometer using CellQuest software. The percentage of cells undergoing apoptosis was analyzed using WINMDI analysis software.

Thymocyte apoptosis will also be analyzed by DNA fragmentation analysis. An aliquot of thymocytes was saved in dry pellet form and stored at -80°C. Total DNA was extracted and subjected to agarose gel electrophoresis (Wani et al. 1999, Chen et al.

2003). DNA fragmentation was then quantified as described by Wani et al. (1999) and Chen et al. (2003).

d. Plasma IgM antibody concentrations

The plasma collected after the primary immunization with SRBC was used to perform and ELISA to detect IgM levels. This assay was performed as described in Temple et al. 1993, with minor modifications. Briefly, SRBC membrane antigen was applied to a 96 well medium binding affinity plate and incubated at 4°C overnight. Plates were then washed 3 times and blocking buffer was applied. Plates were incubated at room temperature for 1hr and then washed 3 times. Samples were applied in duplicate to the plates and then serially diluted. Samples were incubated on the plates for 1.5hr, and then plates were washed 3 times. Affinity purified goat-anti rat HRP conjugated IgM was then applied to all wells and incubated for 1.5hr. The plates were then washed 3 more times and the ABTS substrate was added and plates were incubated at room temperature for 45 minutes. After 45 minutes 3% oxalic acid was added to each well to stop the reaction. Finally, all plates were read on a UV plate reader to determine the intensity of the reaction. ELISA data was then transformed on a Log2 scale for analysis.

Key Research Accomplishments:

**P1 generation**

The protocol for the animal study portion of this research was approved for use by the WPAFB Institutional Animal Care and Use Committee (IACUC) in June 2002 as protocol number **F-WA-2002-0064-A**. In September 2003, approval was obtained from the WPAFB IACUC to expand the study to include 20 pellet treatment groups (See Table 2).

Manufacture of 3,000, 1x2 mm DU pellets was completed by a contractor of the Y-12 National Security Complex in January 2003. NHRC/TD-EHEL took delivery of the 3,000 DU pellets in June 2003.

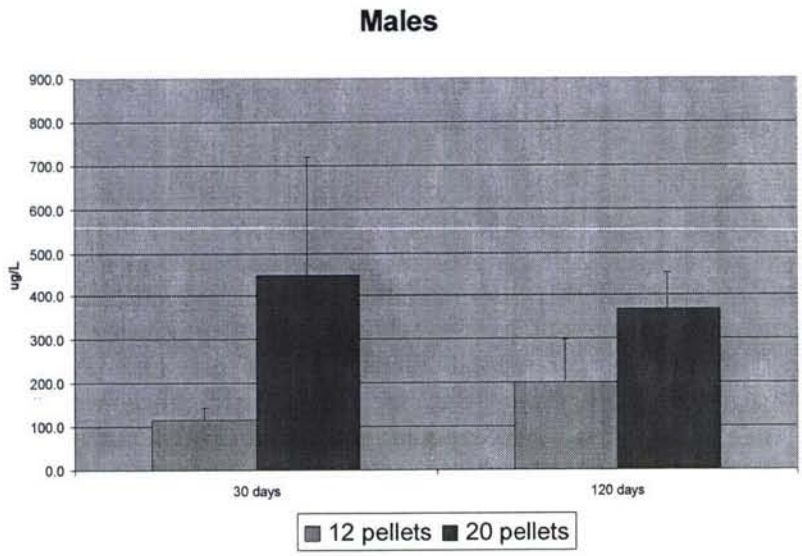
All P1 animals underwent implantation surgery between July and December 2003. A total of 498 P1 adult rats survived the implantation surgery. One female animal from Group 2 (12 Ta steel pellets) died during surgery while under anesthesia. Subsequent investigation determined that the animal was prematurely dosed with the analgesic buprenorphine (0.05 mg/kg) prior to full recovery from isoflurane gas anesthesia and died within minutes of injection. The animal was replaced with another female SD rat from Charles River laboratory derived from a different lot number than the original female animal. No other P1 deaths occurred during surgery or during the 25 day post surgery recovery period.

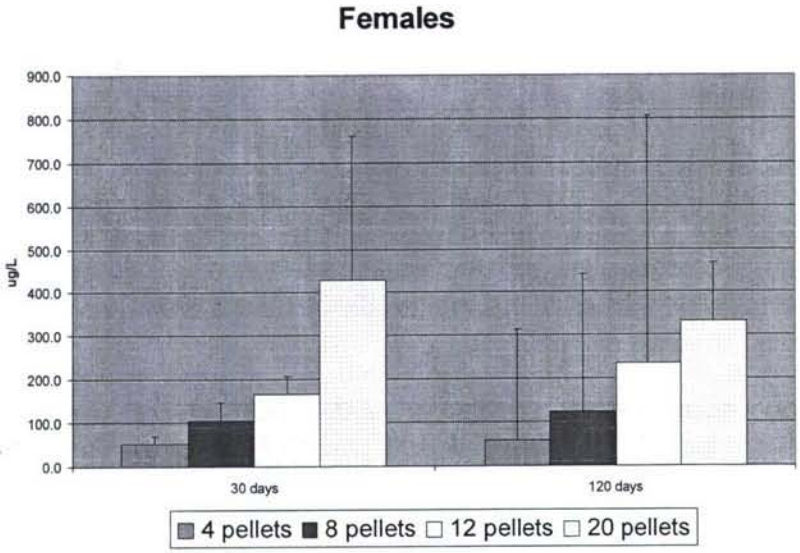
Urinary uranium:

P1 male and female urine uranium concentration was measured on post-implantation days 27 and 117 (Figures 1 and 2). No uranium was detected (LOD: 10 μg/L) in the 24-hour urine output from P1 sham-surgery controls (Group 1) or inert Ta steel implant controls (males: Groups 2, 7-9, 11, 13; females Groups 2, 3, 10, and 13). Uranium was present in the 24 hour urine output of P1 males implanted with 12 DU pellets (Groups 3-6) at an average concentration of 116 ± 27 μg/L (mean ± 95% CI). Uranium was detected in the 24 hour urine output of P1 males implanted with 20 DU pellets (Groups 10 and 12) at an average concentration of 448 ± 272 μg/L (mean ± 95% CI). Uranium was present in the 24 hour urine output of P1 females at an average concentration of 52 ± 17 μg/L, 104 ± 43 μg/L, 165 ± 42 μg/L, and 429 ± 331 μg/L for females implanted with 4, 8, 12, and 20 DU pellets, respectively (Figure 2).

When measured on post-implantation day 117, no uranium was detected (LOD: 10 μg/L) in the 24-hour urine output from P1 sham-surgery controls (Group 1) or inert-controls (males: Groups 2, 7-9, 11, 13; females Groups 2, 3, 10, and 13). Uranium was present in the 24 hour urine output of P1 males implanted with 12 DU pellets at an average concentration of 200 ± 98 μg/L (mean ± 95% CI). Uranium was present in the 24 hour urine output of P1 males implanted with 20 DU pellets at an average concentration of 369

± 84 µg/L (mean ± 95% CI). Uranium was present in the 24 hour urine output of P1 females at an average concentration of 59 ± 254 µg/L, 125 ± 317 µg/L, 235 ± 571 µg/L,



and 334 ± 134 µg/L for females implanted with 4, 8, 12, and 20 DU pellets, respectively (Figure 2).

Uranium in food and water:

No uranium was detected in the rodent chow (LOD: 450 – 550 µg/kg) or drinking water (LOD: 10.0 µg/L) fed to rats throughout the study.

Deaths

Three P1 males died during the 150-day post-implantation surgery period of unknown causes. Of the expired males, one was implanted with 12 DU pellets, one with 20 DU pellets, and one was implanted with 20 Ta steel pellets. Four P1 females died over the 150-day post-implantation period. One sham surgery female died from accidental drowning, another sham surgery female died of unknown causes. One female implanted with 20 DU pellets developed a palpable mammary tumor prior to mating on test day 120 and was euthanized. Gross necropsy confirmed the presence of a mammary gland tumor with the characteristics of a mammary gland carcinoma. One female implanted with 20 DU pellets died during the birthing process on gestation day 22 after mating at 120 days post-implantation (e.g., study day 142). Five underdeveloped fetuses were present in the uterine horn at necropsy. No animals were identified at any time during the 150-day post-implantation surgery period as having outward clinical signs suggestive of toxicity or illness listed in OECD Guideline No. 19 (OECD 2000).

Body Weights/Body Weight Gains:

There were no statistically significant differences in mean body weights and body weight gains of male P1 rats implanted with DU pellets as compared with the mean body weights and body weight gains of male rats implanted with the same number of Ta steel pellets (Table 4). Post-implantation surgery day 150 mean body weights and body weight gains were not available for male rats implanted with 20 DU or 20 Ta steel pellets.

Body weight gains from post-implantation surgery days 0 – 120 were significantly greater for P1 females implanted with 20 DU and 20 Ta steel pellets as compared with the body weight gains over the same time period for sham surgery control P1 females (Table 5). The body weight gains from post-implantation surgery days 0 – 120 for P1 females implanted with 20 DU pellets did not differ significantly from the body weight gain from 0 – 120 days post-implantation for P1 females implanted with 20 Ta steel pellets. Post-implantation surgery day 150 mean body weights and body weight gains were not available for female rats implanted with 20 DU or 20 Ta steel pellets.

Serum Chemistries:

Serum chemistries for a portion of the P1 adults euthanized around 200 days post-implantation surgery are reported in Tables 6 and 7. Although some of the mean serum chemistry values for Groups 2-13 differed significantly for those for Group 1 sham surgery control animals, none of the mean serum chemistry values for P1 rats fell outside the standard serum chemistry reference ranges for the rat.

When compared with Group 1 female values, ALT activities for P1 female rats implanted with 12 Ta steel and 20 DU pellets were significantly ($p \leq 0.05$) lower than for those measured for female sham surgery controls (Table 7). PHOS values for P1 female rats implanted with 20 DU pellets were significantly ($p \leq 0.05$) lower than for those measured for female Group 1 sham surgery controls.

Hematology Values:

The combined hematology values for P1 males and females following necropsy at 200 days post-implantation surgery are reported in Table 8. When assessed using Dunnett's post-hoc test, mean % monocytes were identified as being significantly ($p \leq 0.05$) higher for animals implanted with 20 DU pellets as compared to sham surgery controls; the mean number of platelets x $10^3/\mu L$ were identified as being significantly ($p \leq 0.05$) lower for animals implanted with 20 DU pellets as compared to Group 1 sham surgery controls.

In addition to Dunnett's test, logistic regression was used to analyze internal reference ranges for each parameter generated from sham surgery control hemotology data. Reference ranges were generated by calculating the 20% trimmed range for each parameter listed in Table 8. For each treatment group, counts were made of the number of values falling within and outside the 95% confidence interval of the established reference range for each parameter. The proportion of values falling outside the established reference range was then calculated. Logistic regression was used to test the distributions of "outside the range" and "within the range" between treatment groups.

The regression analysis found that a significantly high proportion of Group 2 (e.g., 12 Ta steel pellets) % monocytes values (87%) and # of granulocyte values (78%) fell outside the established reference ranges for these parameters. A significantly high proportion of Group 10 (e.g., 20 DU pellets) MCV values fell outside the established reference range for MCV (59%).

Uranium Tissue Concentration:

Uranium tissue concentrations were measured for at least 10 P1 animals from each treatment category following euthanasia and necropsy at 200 days post-implantation surgery. For P1 males, uranium was detected in the femur bone, teeth, and kidney in

animals implanted with 12 DU pellets at concentrations of 1.2 ± 9.3, 1.7 ± 1.9, and 5.9 ± 3.4 mg/kg, respectively (mean ± 95% confidence interval). No uranium was detected in any tissue from animals implanted with 12 Ta steel or from sham surgery controls. Tissues from animals implanted with 20 DU or Ta pellets were not available for analysis.

For P1 females, uranium was detected in the femur bone, teeth, and kidney in animals implanted with DU pellets. Uranium was detected in the femur bone at concentrations of 0.81 ± 1.0, 1.6 ± 11.0, and 1.0 ± 3.6 mg/kg, for animals implanted with 4, 8, and 12 DU pellets, respectively (mean ± 95% confidence interval). Uranium was detected in the teeth at a concentration of 1.7 ± 0.6 for animals implanted with 12 DU pellets. No uranium was detected in the teeth from animals implanted with 4 or 8 DU pellets. Uranium was detected in the kidney at concentrations of 1.0 ± 4.7, 5.8, and 3.7 ± 5.0 mg/kg, for animals implanted with 4, 8, and 12 DU pellets, respectively (mean ± 95% confidence interval). No uranium was detected in any tissue from animals implanted with 12 Ta steel or from sham surgery controls. Tissues from animals implanted with 20 DU or Ta pellets were not available for analysis.

Tissue Weights at Necropsy:

Mean absolute P1 tissue weights from animals euthanized and that underwent necropsy 200 days post-implantation surgery are reported in Tables 9 and 10. The mean weight for the sex organs from males implanted with 12 Ta steel pellets or 12 DU pellets were identified as being significantly lower than those for Group 1 sham surgery controls (Table 9). No statistically significant differences in tissue weights were identified for implanted animals versus Group 1 sham surgery controls or animals implanted with 20 Ta steel versus 20 DU pellets.

Relative P1 tissue weights from animals euthanized and that underwent necropsy 200 days post-implantation surgery are reported in Tables 11 and 12. Mean relative heart weights for females implanted with 20 Ta steel pellets were found to be significantly greater than those for Group 1 sham surgery controls. No other statistically significant differences in relative tissue weights were identified for implanted animals versus Group 1 sham surgery controls and for animals implanted with 20 DU pellets versus those implanted with 20 Ta steel pellets.

Histopathology (Col. J. Eggers, Brooks City Base, TX):

Tissues from P1 sham surgery controls, animals implanted with 20 Ta steel pellets, and animals implanted with 20 DU pellets were examined following euthanasia and necropsy at 200 days post-implantation surgery. The tissues examined and the lesions identified in those tissues are summarized in Table 13.

DU implantation sites were characterized by chards of black granular DU material surrounded by a variably thick fibrous capsule and mild to moderate inflammation

composed of mixtures of primarily lymphocytes, plasma cells and macrophages. Many macrophages contained intracytoplasmic black granular pigment (DU material) that had been phagocytosed. Occasionally DU-laden macrophages had migrated further into the muscle away from the original implant site. The one Ta implant area examined contained a thin dense fibrous capsule with minimal inflammation present. Previous chronic (2-yr plus) studies of DU fragments in rat muscle reported a strong correlation with the formation of soft tissue sarcomas. In contrast, none of these three animal's implant sites showed evidence of a proliferative or preneoplastic process in the implant area.

Lesions not attributed to DU toxicity were observed in several tissues. Inflammation and alveolar histiocytosis was a common sporadic finding in the lungs of animals from all treatment groups. In most cases the cause was not evident, but may represent resolved inflammatory foci. In a few animals, the inflammation was suggestive of a foreign body reaction as can occur with inhaled bedding material. When observed, hemorrhage in the lungs was acute and most likely an agonal event associated with euthanasia. Hemosiderin pigment is common finding in most rat spleens. Minimal splenic hemosiderosis was diagnosed if the pigment was easily observed at 2x objective. Small aggregates of inflammatory cells located randomly or in portal areas were commonly observed in the liver of animals from treatment and control groups. These are thought to be due to bacterial showering from the GI tract. Small inflammatory aggregates were occasionally seen in the interstitium of the kidney. Other findings in the kidney included mineralization and changes compatible with early spontaneous degenerative nephropathy common in older rats. The testes were normal in all evaluated sections.

One animal (20 Ta steel pellets) had granulomatous inflammation present in medium sized vessels in several tissues suggesting polyarteritis/polyangitis, a idiopathic disease that is not uncommon in rats.

Sperm motility and concentration:

There was no significant difference ($p \leq 0.05$) in the mean percentage of motile sperm or mean percentage of progressively motile sperm for animals implanted with Ta steel or DU pellets as compared with those for Group 1 sham surgery controls (Table 14). The mean percentage of motile sperm was significantly lower for animals treated by oral gavage with the positive control compound ACH as compared with all other treatment groups. The mean percentage of progressive sperm did not differ for animals treated with ACH as compared with all other treatment groups.

Mean sperm cell tract speed velocity (VCL) was significantly higher ($p \leq 0.001$) for sperm isolated from animals implanted with 20 Ta or 20 DU pellets as compared with the mean VCL for sperm isolated from Group 1 sham surgery animals (Table 14). Mean sperm cell average path velocity (VAP), straight line velocity (VSL), VCL, and lateral sperm head displacement (ALH) were all significantly lower for sperm isolated from animals treated with ACH as compared with sperm isolated from all other treatment groups.

Average caudal sperm concentration for each of the treatment groups is summarized in Table 15. There was no statistically significant ($p \leq 0.05$) difference in average caudal sperm concentration between treatment groups.

Neurobehavioral:

Select P1 animals underwent neurobehavioral assessments beginning 150 days post-implantation. Animals were evaluated in the Spontaneous Locomotor Activity (SLA) Test, Acoustic Startle/Pre-pulse Inhibition (AS/PPI) Test, the Morris Watermaze, and the Conspecific Social Approach Test.

*Acoustic Startle/ Pre-pulse Inhibition:* There were no significant differences in acoustic startle response magnitude or latency to response for P1 male animals implanted with 12 or 20 DU pellets as compared to those for Group 1 sham surgery controls. For P1 females, acoustic startle response magnitude differed significantly by treatment group with magnitude being significantly less than for Group 1 sham surgery controls for females implanted with DU pellets (12 DU pellets < 4 DU pellets <20 DU pellets < 20 Ta steel pellets <sham surgery controls) (Table 16). Acoustic startle response magnitude for females implanted with 12 Ta steel was significantly greater than the response by Group 1 sham surgery controls.

*Spontaneous Locomotor Activity:* For P1 males, no significant differences were found for distance traveled, time resting, ambulatory time, stereotypical behavior/bursts, horizontal movements, or number of rears, when assessed in an open field format. All implanted animals had significantly higher ($p \leq 0.05$) mean number of vertical plane movements as compared with Group 1 sham surgery controls (Table 17).

For P1 females, no significant differences were found for distance traveled, time resting, ambulatory time, stereotypical behavior/bursts, horizontal movements, vertical plane movements or number of rears, when assessed in an open field format.

*Morris Watermaze*: For both the males and the females, there were no significant differences in the distance swam before "finding" the watermaze platform or time to find the watermaze platform when compared among treatment groups.

*Conspecific Social Approach Test:* The mean coincidence contact frequency for P1 females was found to be significantly different for all groups (ANOVA, $p \leq 0.05$) (Table 18). Coincident contact frequency was elevated for females implanted with 20 Ta steel pellets, however, post-hoc tests were not carried out to determine if the mean contact frequency for this group differed significantly from that of the sham surgery controls.

Immune function:

Evidence for significant immune function suppression was apparent for CYA-treated animals as compared to the immune function of P1 Group 1 sham surgery controls. The DTH stimulation indices and IgM titer were significantly lower for CYA-treated animals as compared to P1 Group 1 sham surgery controls. The thymocyte apoptotic index was significantly higher for CYA-treated animals as compared to P1 Group 1 sham surgery controls. Relative thymus mass and thymocyte viability were not compared between CYA-treated animals and P1 Group 1 sham surgery controls.

There were no significant differences for thymocyte viability or total thymus cellularity for P1 treatment Groups 2-13 when compared to Group 1 sham surgery controls. Further analysis determined that female sex was a significant factor for both thymocyte viability and total thymus cellularity. The differences between Groups 2-13 and Group 1 were re-analyzed for males and females separately. The differences were not statistically significant when re-analyzed by sex.

**F1a Generation**

Mating success:

There were no significant differences in mating success rates or average insemination times for P1 males when compared between the 5 treatment groups listed in Table 19. Percent mating success was lower for Group 1 sham surgery controls as compared with the other 4 treatment groups, but this difference was not statistically significant.

Gestation length and weight gain:

The length of gestation and gestation weight gain of P1 females impregnated 30-45 days post-implantation surgery are listed in Table 20. No significant differences were found for gestation length or gestation weight gain for animals implanted with 12 Ta steel, or 4, 8, or 12 DU pellets as compared to Group 1 sham surgery controls. No significant differences were found for gestation length or gestation weight gain for animals implanted with 20 Ta steel pellets versus animals implanted with 20 DU pellets.

Litter size, pup sex ratio, and pup survival:

Litter size, percentage of males per litter, and pup survival for litters born to mothers mated at 30-45 days post-implantation day surgery are summarized in Table 21. ANOVA tests were not significant for any of the parameters listed in Table 21. Multiple comparison tests (Dunnett's test) for each parameter versus Group 1 sham surgery controls found no statistically significant differences for any of the 13 mating groups.

PND 4 and PND 20 pup uranium content:

No uranium was detected in any whole body homogenates from approximately 600 PND4 pups derived from P1 parents of treatment groups 1-13. The number of PND4 pups analyzed for uranium content was about 20% of the total number of pups that survived until PND4. The LOD of uranium in PND4 whole body homogenates ranged from 450-600 µg/kg and was inversely dependent on the mass of the PND4 pup.

No uranium was detected in any whole body homogenates from approximately 400 PND20 pups derived from P1 parents of treatment groups 1-13. The number of PND20 pups analyzed was about 25% of the total number of pups that survived until PND20. The LOD of uranium in PND20 whole body homogenates was 420 – 550 µg/kg.

No uranium was detected in any of the pooled tissues from approximately 250 PND20 pups derived from P1 parents of treatment groups 1-13. Uranium analysis was carried out on homogenates of the following tissues: liver, kidneys, brain, spleen, thymus, testes, ovaries, GI tract, and femur.

Abnormalities:

Visual inspection of pups through PND1-4 and gross necropsy of approximately 20% of all pups alive at PND20 did not find evidence of abnormalities in appearance or physical abnormalities of the major organs (excluding brain and spinal column). Two litters were born with hydrocephalus characterized by proportionally small bodies and proportionally large heads. One litter was born to Group 1 (e.g., sham surgery) parents and the other was born to Group 2 (high dose Ta steel) parents. No other outward abnormalities were observed for the approximately 2,000 pups allowed to survive to PND20.

One hundred thirty rib cages (10 per dose group) from pups born to mothers mated at 30-45 days post-implantation day surgery were evaluated for abnormalities following established guidelines (M.S. Christian in *Principles and Methods of Toxicology-Fourth Edition*, edited by A. Wallace Hayes, 2001, Philadelphia, PA, pp. 1301-1381). Rib cages were evaluated independently by a trained veterinary pathologist (MAJ Mary Cooper, Brooks City Base, TX) and two study technicians with experience in animal toxicology studies. All persons involved with evaluating the rib cages were blinded to the pup's treatment status. No cases of missing ribs were identified for pups born to mothers mated at 30-45 days post-implantation day surgery. One case of an extra rib (13 vs 14) occurred in a rib cage from a pup born to Group 2 P1 parents (12 Ta steel pellets). No cases of rib waviness, branching, fusion, misalignment, or thickened areas of ossification were identified. There was 100% agreement on the findings for each rib cage evaluated between the study pathologist and technicians involved in independent rib cage evaluations.

Neurobehavioral:

*Maternal care/retrieval:* Mean maternal retrieval latencies (e.g., average time to find F1a pup and retrieve to nest) for each P1 treatment group tested on F1a PND2 is reported in Table 22. Maternal retrieval times for P1 mothers implanted with 20 DU pellets (Groups 10 and 13) were significantly ($p \leq 0.05$) lower than the mean retrieval time for Group 11 (20 Ta steel pellets).

*Righting latency*: The results for the righting latency tests conducted on PND4 are summarized in Table 23. There were no significant differences for pups born to parents from treatment Groups 2-13 as compared to pups born to parents of the sham surgery control group. There were no significant differences for pups born to parents from treatment Group11 as compared to pups born to parents of treatment groups 10, 12, and 13.

*Pup separation distress:* F1a pup vocalization frequency was measured in response to being separated from their mother on PND7. Vocalization frequency in response to separation was significantly lower for F1a pups from P1 treatment Group 5 as compared to pups derived from sham surgery control parents (Table 24). There were no significant

differences in vocalization frequency for F1a pups from P1 treatment groups 10, 12, and 13 as compared to the mean vocalization frequency for F1a pups derived from P1 20 Ta steel pellet inert controls (Group 11).

*Juvenile Play:* The results for the F1a PND17-32 juvenile play (dorsal contacts, pins, and pin latency) testing are summarized in Tables 25, 26, and 27. There were no significant differences in measures of PND17-32 juvenile play (dorsal contacts, pins, and pin latency) for F1a pups born to parents from treatment Groups 2-13 as compared to pups born to parents of the sham surgery control group. There were no significant differences for F1a pups born to parents from treatment Group11 as compared to pups born to parents of treatment groups 10, 12, and 13.

*Spontaneous Locomotor Activity:* F1a spontaneous locomotor activity was assessed on PND 32 (Tables 28-36). No significant differences were found for pups from parental Groups 2-9 versus Group 1 sham surgery controls for time ambulatory, distance traveled, stereotypical behavior/bursts, horizontal movements, vertical movements, ambulatory movements, or number of rears, when assessed in an open field format.

Significant ($p \leq 0.05$) differences were found for time resting and stereotypical behavior when Group 10 (parents implanted with 20 DU pellets only) was compared with Group 11 (parents implanted with 20 Ta steel pellets only). Significant ($p \leq 0.05$) differences were found for distance traveled, stereotypical bursts, horizontal movements, and number of rears when Group 13 (female parents implanted with 20 DU pellets) was compared with Group 11 (parents implanted with 20 Ta steel pellets only).

*Acoustic Startle/ Pre-pulse Inhibition:* There were no significant differences in acoustic startle response magnitude or latency to response for F1a pups when assessed PND45-47 (Tables 37 and 38).

*Morris Watermaze*: F1a pup learning and memory was evaluated in the Morris Watermaze at PND59-63. There were no significant differences in the distance swam before "finding" the watermaze platform or time to find the watermaze platform when compared among the treatment groups (Tables 39 and 40).

Hematology Values:

The combined hematology values for F1a male and female pups euthanized at PND90 reported in Table 41. When assessed using Dunnett's post-hoc test, MCV was identified as being significantly ($p \leq 0.05$) lower for animals from treatment groups 3, 4, 8, and 9 as compared to the mean MCV for F1a pups derived from parents of the sham surgery controls treatment group. Mean MCV for animals derived from treatment Group 10 was significantly ($p \leq 0.05$) lower for animals from treatment group 11. The mean WBC concentration for animals derived from treatment Group 10 was significantly ($p \leq 0.05$) higher than for animals from treatment Group 11.

Several parameters were associated with significantly high proportions of values falling outside the 20% trimmed means for Group 1 parameters. A significant number of % lymphocyte values for Group 4 and Group 6 fell outside the 20% trimmed mean for Group 1. A significant number of MCV concentration values for Group 2, 3, 4, 8, and 9 fell outside the 20% trimmed mean for Group 1. A significant number of MCH concentration values for Group 3 and 6 fell outside the 20% trimmed mean for Group 1. A significant number of MCHC concentration values for Group 5 and 9 fell outside the 20% trimmed mean for Group 1.

F1a PND90 sperm motility and concentrations:

F1a PND 90 sperm motility and concentration were measured and compared between male offspring originating from sham surgery controls versus those originating from one of the 4 categories of P1 pellet implantation. No statistically significant differences were found for % motile sperm, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), amplitude of lateral sperm head displacement (ALH), and sperm head beat-cross frequency (BCF) when these parameters for F1a pups when compared between P1 treatment category (Table 42).

Average caudal sperm concentration for each of the treatment groups is summarized in Table 43. There was no statistically significant ($p \leq 0.05$) difference in average caudal sperm concentration from F1a groups 2-9 when compared with F1a pups derived from sham surgery control parents. Average caudal sperm concentration for F1a groups 11, 12, and 13 did not differ significantly from the average caudal sperm concentration for F1a group 10.

Pup adult stage weight gain and survival:

A select number of F1a pups were housed separately until PND90 to study their development and survival through early adulthood. The average body weights and body weight gains of F1a pups for which both PND20 and PND50 body weights were available are listed in Tables 44 and 45. No significant differences were found for average PND20 or PND50 body weights or body weight gains for pups born to parents from treatment groups 2-9 versus those derived from Group 1 sham surgery control parents. There were no body weight data available post PND20 for pups derived from parents from treatment groups 10-13.

Of the select F1a animals studied through PND90, no animals died prematurely prior to necropsy.

Immune function:

Evidence for significant immune function suppression was apparent for CYA-treated animals as compared to the immune function of F1a pups derived from Group 1 sham surgery controls. The DTH stimulation indices, IgM titer, and relative thymus mass were significantly lower for CYA-treated animals as compared to F1a Group 1 pups. There was no significant difference in % thymocyte viability for CYA-treated animals as compared to F1a Group 1 pups.

There were no statistically significant differences in DTH stimulation indices, IgM titers, and relative thymus mass for F1a Groups 2-9 when compared to the results for F1a Group 1. Thymus cell apoptosis was increased significantly in Groups 10-13 F1a pups suggesting that increased thymocyte apoptosis was associated with parental implantation of 20 pellets regardless of the chemical composition. Since both DU and Ta treatments were elevated, it is likely that this effect may have been due to pellet burden stress felt by the parents. However, there were no significant differences noted in the percentage of viable thymocytes for F1a Group 2-13 pups as compared to F1a Group 1 pups.

**F1b Generation**

Mating success:

When compared between mating periods, the success rate at 30-45 days post-implantation (e.g., F1a generation) for the 13 treatment groups was lower as compared with the mating success rate for the 120-145 days post-implantation mating period (86% versus 91%). However, this difference was not statistically significant.

P1 reproductive success at 120-145 days post-implantation is summarized in Table 46. There were no significant differences in mating success rates or average insemination times when compared between the 5 P1 treatment categories.

Gestation length and weight gain:

The length of gestation and gestation weight gain of P1 females impregnated 30-45 days post-implantation surgery are listed in Table 47. The gestation lengths for females implanted with 8 or 20 DU pellets was found to be significantly shorter than for Group 1 sham surgery controls. The gestation length for females implanted with 20 DU pellets did not differ significantly for females implanted with 20 Ta steel pellets.

No significant differences were found for gestation weight gain among treatment groups when compared against the gestation weight gains for sham surgery controls or to the gestation weight gain for females implanted with 20 Ta steel pellets (inert implant controls).

Litter size, pup sex ratio, and pup survival:

Litter size, percentage of males per litter, and pup survival for litters born to mothers mated at 120-145 days post-implantation day surgery are summarized in Table 48. ANOVA tests were not significant for any of the parameters listed in Table 48. The mean for each parameter for treatment groups 2-13 were compared to Group 1 sham surgery controls using Dunnett's multiple comparisons test. No significant differences were found for any of the parameters evaluated in the Dunnett's multiple comparisons test.

ANOVA tests for Groups 11-13 were significant ($p \leq 0.05$) for percent males at PND1. Percent males at PND1 for Group 12 was found to be significantly lower than for the percent males at PND1 for Group 11 (parents implanted with 20 Ta steel pellets only) when Groups 10-13 were compared using Dunnett's multiple comparisons test.

F1b average litter size and weight are reported in Table 49. ANOVA tests were not significant for average litter size or weight at PND1 or PND4 for Groups 1-13. PND1 and PND4 average litter size and weight for Groups 1-13 were compared to sham surgery

controls using Dunnett's multiple comparisons test. No significant differences were found for any of the parameters evaluated in the Dunnett's multiple comparisons test. ANOVA tests for Groups 11-13 were significant ($p \leq 0.05$) for average PND4 weight. Average PND4 weight for Group 12 was found to be significantly lower than for the average PND4 weight for Group 11 (parents implanted with 20 Ta steel pellets only) when Groups 10-13 were compared using Dunnett's multiple comparisons test.

Average male and female pup weight gains from PND5-PND20 are reported in Table 50. There were no significant differences in PND5-PND20 average pup weight gain for parental treatment groups 2-13 when compared with the average PND5-PND20 weight gain for pups derived from Group 1 sham surgery controls. PND5-PND20 average pup weight gain for parental treatment groups 10, 12, and 13 were not significantly different from PND5-PND20 average pup weight gain for Group 11.

PND 4 pup uranium content:

No uranium was detected in any whole body homogenates from approximately 600 PND4 F1b pups derived from P1 parents of treatment groups 1-13. The number of PND4 F1b pups analyzed for uranium content was about 20% of the total number of pups that survived until PND4. The LOD of uranium in PND4 F1b pup whole body homogenates ranged from 450-600 μg/kg and was inversely dependent on the mass of the PND 4 pup.

Abnormalities:

Visual inspection of F1b pups through PND1-4 and gross necropsy of approximately 20% of all F1b pups alive at PND 20 did not find evidence of abnormalities in appearance or physical abnormalities of the major organs (excluding brain and spinal column).

One hundred sixty-eight rib cages (10+ per dose group) from F1b pups were evaluated for abnormalities following established guidelines (M.S. Christian in *Principles and Methods of Toxicology-Fourth Edition*, edited by A. Wallace Hayes, 2001, Philadelphia, PA, pp. 1301-1381). Rib cages were evaluated independently by a trained veterinary pathologist (MAJ Mary Cooper, Brooks City Base, TX) and two study technicians with experience in animal toxicology studies. All persons involved with evaluating the rib cages were blinded to the pup's treatment status. All rib cages evaluated had the correct number of ribs expected for the SD rat (n=13). No cases of rib waviness, branching, fusion, misalignment, or thickened areas of ossification were identified. There was 100% agreement on the findings for each rib cage evaluated between the study pathologist and technicians involved in independent rib cage evaluations.

Neurobehavioral:

*Maternal care/retrieval:* Mean maternal retrieval latencies (e.g., average time to find F1b pup and retrieve to nest) for each P1 treatment group tested on F1b PND 2 is reported in Table 51. Maternal retrieval times for P1 mothers implanted with 20 DU pellets (Groups 10 and 13) were significantly ($p \leq 0.05$) lower than the mean retrieval time for Group 11 (20 Ta steel pellets).

*Righting latency*: The results for the righting latency tests conducted on PND4 are summarized in Table 52. There were no significant differences for pups born to parents from treatment Groups 2-13 as compared to pups born to parents of Group 1 sham surgery controls. There were no significant differences for pups born to parents from treatment Group 11 as compared to pups born to parents of treatment groups 10, 12, and 13.

*Pup separation distress:* F1b pup vocalization frequency was measured in response to being separated from their mother on PND 7 (Table 53). There were no significant differences in vocalization frequency for F1b pups from P1 treatment Groups 2-13 as compared with the vocalization frequency for F1b pups from P1 treatment Groups 1. There were no significant differences in vocalization frequency for F1b pups from P1 treatment Groups 10, 12, and 13 as compared to the mean vocalization frequency for F1b pups derived from P1 20 Ta steel pellet inert controls (Group 11).

*Juvenile Play:* The results for the F1b PND17-32 juvenile play (dorsal contacts, pins, and pin latency) testing are summarized in Tables 54, 55, and 56. There were no significant differences in measures of PND17-32 juvenile play (dorsal contacts, pins, and pin latency) for F1b pups born to parents from treatment Groups 2-13 as compared to pups born to parents of the sham surgery control group. There were no significant differences for F1b pups born to parents from treatment Group11 as compared to pups born to parents of treatment groups 10, 12, and 13.

*Spontaneous Locomotor Activity:* F1b spontaneous locomotor activity was assessed on PND 32 (Tables 57-65). No significant differences were found for pups from parental Groups 2-9 versus Group 1 sham surgery controls for time ambulatory, distance traveled, stereotypical behavior/bursts, horizontal movements, vertical movements, ambulatory movements, or number of rears, when assessed in an open field format. Additionally, there were no significant differences for F1b pups born to parents from treatment Group 11 as compared to pups born to parents of treatment groups 10, 12, and 13.

*Acoustic Startle/ Pre-pulse Inhibition:* There were no significant differences in acoustic startle response magnitude or latency to response for F1b pups when assessed PND45-47 (Tables 66 and 67).

*Morris Watermaze*: F1b pup learning and memory was evaluated in the Morris Watermaze at PND59-63. F1b pups born to parents of Group 10 had significantly faster times to find the platform in the watermaze as compared with the F1b pups born to sham

surgery control parents (Table 68). However, F1b pups born to sham surgery control parents had the longest times to find the platform in the watermaze test. There were no significant differences in the distance swam before "finding" the watermaze platform for F1b pups when compared by parental treatment group (Table 69).

F1b PND20 pup whole body uranium concentrations:

No uranium was detected in any whole body homogenates from approximately 400 F1b PND 20 pups derived from P1 parents of treatment groups 1-13. The number of F1b PND 20 pups analyzed was about 25% of the total number of pups that survived until PND 20. The LOD of uranium in F1b PND 20 whole body homogenates was 420 – 550 μg/kg.

Pup adult stage weight gain and survival:

A select number of F1b pups were housed separately until PND200 to study their development and survival through early adulthood. The average body weights and body weight gains of male F1b pups are reported in Tables 70 and 71. There were no significant differences in male body weight or body weight gain for Groups 2-9 as compared with Group 1 sham surgery controls. Also, there were no significant differences in body weight or body weight gain for Group 10 versus Group 11 pups. PND120 body weights for Group 10 and 12 male pups were not available because of errors made in data collection.

The average body weights and body weight gains of female F1b pups are reported in Tables 72 and 73. PND60 body weights for Groups 4, 8, and 9 were significantly larger than the PND60 body weights for pups born to Group 1 sham surgery controls. Average PND90 weights for female Group 10 pups were significantly lower than the PND90 body weights for female pups born to Group 1 sham surgery controls. Additionally, PND20-60 body weight gains were significantly higher for Group 9 pups as compared to pups born to sham surgery controls. Group 10 PND20-60 mean weight gain was significantly lower than the PND20-60 mean weight gain for pups derived from parents implanted with 20 Ta steel pellets (Group 11). PND120 body weight for Group 12 pups was not available because of errors made in data collection.

Of the 479 F1b animals studied through PND200, 8 animals died prematurely prior to necropsy. Two Group 6 pups (1 male and 1 female) died shortly after separation from their mother on PND20. Both animals were underweight for their age. One Group 2 male, 1 Group 5 male, 1 Group 6 male, and 3 Group 9 pups (2 males, 1 female) were found dead of unknown causes. None of the pups were derived from the same P1 mating pair.

F1b PND200 pup tissue and organ uranium concentrations:

The uranium content of liver, kidneys, brain, spleen, thymus, testes, ovaries, GI tract, and the femur was measured for F1b PND200 pups derived from P1 parents of treatment groups 1-13. Uranium was detected in the pooled GI tract tissues (stomach, small intestine, large intestine) from Group 2 F1b PND 200 pups at a concentration of 51 µg/kg (LOD: 50 µg/kg). Uranium was not detected in any other pooled tissues from the approximately 250 F1b PND200 pups analyzed (LOD: 50 µg/kg).

Hematology Values:

The combined hematology values for F1b males and females following necropsy at PND 200 are reported in Table 74. When assessed using Dunnett's post-hoc test, the mean WBC concentration for Group 10 was significantly higher than the mean WBC concentration for Group 11. Also, the mean MCHC concentration for Group 10 was significantly lower than the mean MCHC concentration for Group 11.

In addition to Dunnett's test, logistic regression was used to analyze internal reference ranges for each parameter generated from sham surgery control hemotology data. Reference ranges were generated by calculating the 20% trimmed range for each parameter listed in Table 74. For each treatment group, counts were made of the number of values falling within and outside the 95% confidence interval of the established reference range for each parameter. The proportion of values falling outside the established reference range was then calculated. Logistic regression was used to test the distributions of "outside the range" and "within the range" between treatment groups.

The regression analysis found that a significantly high proportion of Group 3 (73%), 5 (67%), 10 (80%), and 11 (89%) RBC concentration values fell outside the established reference range for this parameter.

F1b PND200 sperm motility and concentrations:

F1b PND200 sperm motility and concentration were measured and compared between male offspring originating from sham surgery controls when compared by paternal treatment group (Table 75). No statistically significant differences were found for % motile sperm, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), amplitude of lateral sperm head displacement (ALH), and sperm head beat-cross frequency (BCF) when these parameters for F1b pups when compared between P1 treatment category.

Average caudal sperm concentration for each of the treatment groups is summarized in Table 76. There was no statistically significant ($p \leq 0.05$) difference in average caudal sperm concentration from F1b parental treatment Groups 2-9 when compared with F1b pups derived from sham surgery control parents (Group 1). Average caudal sperm

concentration for F1b Groups 10, 12, and 13 did not differ significantly from the average caudal sperm concentration for F1b Group 11.

Tissue Weights at Necropsy:

Mean absolute and relative F1b male tissue weights from animals euthanized and that underwent necropsy 200 days post-implantation surgery are reported in Tables 77 and 78. There were no significant differences in mean absolute or relative tissue weights for Groups 2-9 when compared against the mean absolute or relative tissue weights for Group 1. There were no significant differences in mean absolute or relative tissue weights for Groups 10, 12, or 13 when compared against the mean absolute or relative tissue weights for Group 11.

Mean absolute and relative F1b female tissue weights from animals euthanized and that underwent necropsy 200 days post-implantation surgery are reported in Tables 79 and 80. There were no significant differences in mean absolute tissue weights for Groups 2-9 when compared against the mean absolute or relative tissue weights for Group 1. Group 10 mean relative liver and heart weights and Group 11 mean relative heart weights were significantly greater than those for Group 1. However, there were no significant differences in mean absolute or relative tissue weights for Groups 10, 12, or 13 when compared against the mean absolute or relative tissue weights for Group 11.

Immune function:

Evidence for significant immune function suppression was apparent for CYA-treated animals as compared to the immune function of F1b pups derived from Group 1 sham surgery controls. The DTH stimulation indices, IgM titer, and relative thymus mass were significantly lower for CYA-treated animals as compared to F1b Group 1 pups. There was no significant difference in % thymocyte viability for CYA-treated animals as compared to F1b Group 1 pups.

There were no statistically significant differences in DTH stimulation indices, IgM titers, relative thymus mass, and % thymocyte viability for F1b Groups 2-13 as compared to the results for these measurements for F1b Group 1.

**F2 Generation**

Mating success:

A total of 261 matings were performed with 179 pregnancies produced. The overall mating success rate of F1b mates regardless of treatment type was 69%. This rate was significantly lower than the mating success rate for P1 rats when mated at 30-45 days post-implantation (86%) and 120-145 days post-implantation (91%). Based on historical control data from mating studies carried out at Charles River, the mating success rate for SD rats ranges from 80 – 100% for SD rats ranging in age from 11 – 16 weeks of age. The possible reasons for the low mating success for F1b rats are not readily apparent. The failure rate was random over the F1b mating period (Spring – Fall 2004) and did not occur at increased rates at any particular time during the mating period. No significant treatment related effects were identified on sperm motility or concentration of F1b animals or neurobehavior. The lack of a treatment effect suggests that mating success may have been negatively affected by unidentified environmental factors (e.g., temperature, light:dark cycles, animal room overcrowding, etc).

There were no significant differences in mating success rates among the 13 F1b treatment groups (Table 81). There were no significant differences in average mating success rates for Groups 2-13 when compared with the average mating success rate for Group 1 animals derived from sham surgery controls parents. There were no significant differences in average mating success rates for Groups 10, 12, and 13 when compared with the average mating success rate for Group 11 animals derived from parents implanted with 20 Ta steel pellets.

Gestation length and weight gain:

The length of gestation and gestation weight gain of impregnated F1b females are listed in Table 82. Gestation length was compared using survival analysis, loglinear regression analysis, and regression analysis. There was an overall significant difference among groups for the survival analysis ($P = 0.0114$), but not for the loglinear ($P = 0.0822$) or regression ($P = 0.0718$) analyses. The three methods agree that Group 11 average gestation length was significantly lower than the average gestation length for Group 1. The three methods agree that the average gestation length Group 10 is significantly greater than the average gestation length for Group 11.

For gestation weight gain, there was no significant overall differences by the Kruskal-Wallis test ($P = 0.78$), ANOVA on ranks ($P = 0.795$), or parametric ANOVA ($P = 0.919$). The average gestation weight gain for Groups 2-13 did not differ significantly from the average gestation weight gain for Group 1 when compared by Dunnett's test. The average gestation weight gain for Group 11 did not differ significantly from the average gestation weight gain for Group 10 when compared by Dunnett's test.

Litter size, pup sex ratio, and pup survival:

Litter size, percentage of males per litter, and pup survival for litters born to F1b mothers mated at PND70 are summarized in Table 83. Differences between treatment Groups 2-13 versus Group 1 sham surgery controls were not statistically significant when compared using ANOVA or Dunnett's multiple comparisons test. There were no significant differences for any parameter measured between Group 11 versus treatment Groups 10, 12, and 13 when compared using ANOVA or Dunnett's multiple comparisons test.

F2 average litter size and weight at PND1 and PND4 are reported in Table 84. ANOVA tests were not significant for average litter size or weight at PND1 or PND4 for Groups 1-13 indicating that there were no significant differences for these parameters across treatment groups. Also, the differences for these parameters for Groups 2-13 versus Group 1 where not statistically significant when compared using Dunnett's multiple comparisons test. Also, there were no differences found when Groups 10, 12, and 13 were compared to Group 11 using Dunnett's multiple comparisons test.

F2 male and female pup average weight gains from PND5-20 for each treatment group were compared using ANOVA and Dunnett's multiple comparisons test (Table 85). There were no significant differences for average PND5-20 weight gain for Groups 2-13 when compared to the average PND5-20 weight gain for group 1 sham surgery controls. There were no significant differences for average PND5-20 weight gain for treatment Groups 10, 12, and 13 compared to the average PND5-20 weight gain for Group 11.

Abnormalities:

Visual inspection of F2 pups through PND 1-4 and gross necropsy of approximately 20% of all F2 pups alive at PND 20 found no evidence of abnormalities in appearance or physical abnormalities of the major organs (excluding brain and spinal column).

Two hundred seventeen rib cages (15+ per dose group) from F2 pups were evaluated for abnormalities following established guidelines (M.S. Christian in *Principles and Methods of Toxicoloy-Fourth Edition*, edited by A. Wallace Hayes, 2001, Philadelphia, PA, pp. 1301-1381). Rib cages were evaluated independently by two technicians with experience in animal toxicology studies. All persons involved with evaluating the rib cages were blinded to the pup's treatment status. All rib cages evaluated had the correct number of ribs expected for the SD rat (n=13). No cases of rib waviness, branching, fusion, misalignment, or thickened areas of ossification were identified. There was 100% agreement on the findings for each rib cage evaluated between the study pathologist and technicians involved in independent rib cage evaluations.

Pup adult stage weight gain and survival:

A select number of F2 pups were housed separately until PND90 to study their development and survival through early adulthood. The average body weights and body weight gains of male F2 pups through PND90 are reported in Tables 86 and 87. There were no significant differences in male body weight or body weight gain for Groups 2-9 as compared with Group 1. Also, there were no significant differences in body weight or body weight gain for Group 10 versus Group 11 pups.

The average body weights and body weight gains of female F2 pups are reported in Tables 88 and 89. There were no significant differences in male body weight or body weight gain for Groups 2-9 as compared with Group 1. Also, there were no significant differences in body weight or body weight gain for Group 10 versus Group 11 pups.

Of the 327 F2 animals studied through PND90, 3 animals died prematurely prior to necropsy. One Group 5 female and one Group 11 male flooded their cages and drowned. One Group 4 female died of unknown causes.

Hematology Values:

The combined hematology values for F2 males and females following necropsy at PND90 are reported in Table 90. When assessed using Dunnett's multiple comparisons test, the mean % monocytes for Group 4 and Group 6 were significantly higher than the % monocytes for Group 1. The mean % hematocrit for Group 11 was found to be significantly higher and the mean MCV for Group 11 was found to be significantly lower than for Group 1 when compared using Dunnett's multiple comparisons test. The MCHC concentration for Group 10 was found to be significantly lower than the concentration for Group 11 when compared using Dunnett's multiple comparisons test.

In addition to Dunnett's test, logistic regression was used to analyze internal reference ranges for each parameter generated from sham surgery control hemotology data. Reference ranges were generated by calculating the 20% trimmed range for each parameter listed in Table 90. For each treatment group, counts were made of the number of values falling within and outside the 95% confidence interval of the established reference range for each parameter. The proportion of values falling outside the established reference range was then calculated. Logistic regression was used to test the distributions of "outside the range" and "within the range" between treatment groups.

The regression analysis found none of the parameters in Table 90 had a significantly high proportion of values outside the 20% trimmed reference ranges.

F2 PND90 sperm motility and concentrations:

F2 PND90 sperm motility and concentration were measured and compared between male offspring originating from sham surgery controls when compared by paternal treatment group (Table 91). No statistically significant differences were found for % motile sperm, straight line velocity (VSL), average path velocity (VAP), amplitude of lateral sperm head displacement (ALH), and sperm head beat-cross frequency (BCF) when these parameters for F2 pups when compared between P1 treatment category. Group 2 sperm curvilinear velocity (VCL) was found to be significantly higher than the VCL for Group 1. For Group 10, % progressive sperm was significantly higher as compared to Group 11. However, % progressive sperm for Group 11 was found to be elevated as compared to Group 1 ($p \leq 0.086$). Group 10 % progressive sperm was not significantly different for the % progressive sperm for Group 1 ($p = 1.00$).

Average caudal sperm concentration for each of the treatment groups is summarized in Table 92. There was no statistically significant ($p \leq 0.05$) difference in average caudal sperm concentration from F2 parental treatment groups 2-9 when compared with F2 pups derived from sham surgery control parents (Group 1). Average caudal sperm concentration for F2 groups 10, 12, and 13 did not differ significantly from the average caudal sperm concentration for F2 group 11.

Tissue Weights at Necropsy:

Mean absolute and relative F2 male tissue weights from animals euthanized and that underwent necropsy 90 days post-implantation are reported in Tables 93 and 94. Absolute mean heart weight for Group 6 was found to be significantly greater than the absolute mean heart weight for Group 1 (Table 93). Group 10 mean relative kidney weight was significantly lower than the mean relative kidney weight for Group 11 (Table 94). There were no other significant differences in mean absolute or relative tissue weights when Groups 2-11 where compared to Group 1 or when Groups 10, 12, and 13 were compared with Group 11.

Mean absolute and relative F2 female tissue weights from animals euthanized and that underwent necropsy 90 days post-implantation are reported in Tables 95 and 96. Absolute mean heart weight for Group 8 was found to be significantly greater than the absolute mean heart weight for Group 1 (Table 95). Relative mean kidney weights and brain weights of Group 10 were significantly greater than those for Group 11 (Table 96). Both relative mean kidney weights and brain weights of Group 10 and 11 were not significantly different from those for Group 1.

No other differences were found to be significant when mean absolute and relative tissue weights for Groups 2-11 were compared with those for Group 1 or when the means for Groups 10, 12, and 13 were compared with those for Group 11.

Immune function:

F2 immune function tests have not been completed as of 1 June 2005.

| Table 1: Parameters measured for P1, F1a, F1b, and F2 generations | |
|---|---|
| Generation | Parameter |
| P1 | Urinary uranium concentration |
| | Body weight |
| | Body weight gain |
| | Mating success |
| | Gestation weight gain |
| | Gestation length |
| | F1 PND1 litter size |
| | F1 PND1 litter weight |
| | F1 Male:female pup ratio |
| | Maternal care of young |
| | Immune function |
| | Neurobehavior |
| | Survival to 150 days post-implantation |
| | Post-necropsy tissue weights |
| | Post-necropsy tissue uranium concentration |
| | Hematology |
| | Serum chemistries |
| | Sperm concentration and motility |
| F1a | PND4 litter size |
| | PND4 litter weight |
| | Gross malformations, PND1-4 |
| | Survival, PND1-4 |
| | PND5 weight |
| | Pup neurobehavior, PND5 |
| | PND5-20 survival |
| | PND5-20 weight gain |
| | Body weight, PND20 |
| | PND20 whole body uranium concentration |
| | PND20 tissue uranium concentration |
| | Pup neurobehavior, PND20-70 |

| Table 1: Parameters measured for P1, F1a, F1b, and F2 generations | |
|---|---|
| Generation | Parameter |
| | Body weight, PND50, 60, 90, 120 |
| | Body weight gain, PND20-50, 20-60, 20-90, 20-120. |
| | Immune function |
| | Survival to PND90 |
| | Post-necropsy tissue weights |
| | Hematology |
| | Serum chemistries |
| | Sperm concentration and motility |
| F1b | PND4 litter size |
| | PND4 litter weight |
| | Gross malformations, PND1-4 |
| | Survival, PND1-4 |
| | PND5 weight |
| | Pup neurobehavior, PND5 |
| | PND5-20 survival |
| | PND5-20 weight gain |
| | Body weight, PND20 |
| | PND20 whole body uranium concentration |
| | PND20 tissue uranium concentration |
| | Pup neurobehavior, PND20-70 |
| | Body weight, PND60, 90, 120 |
| | Body weight gain, PND20-60, 20-90, 20-120. |
| | Immune function |
| | Survival to PND 200 |
| | Post-necropsy tissue weights |
| | Hematology |
| | Serum chemistries |
| | Sperm concentration and motility |
| | Mating success, F2 generation |

| Table 1: Parameters measured for P1, F1a, F1b, and F2 generations | |
|---|---|
| Generation | Parameter |
| | Gestation weight gain |
| | Gestation length |
| | F2 PND1 litter size |
| | F2 PND1 litter weight |
| | F2 Male:female pup ratio |
| | Maternal care of young |
| F2 | PND4 litter size |
| | PND4 litter weight |
| | Gross malformations, PND1-4 |
| | Survival, PND1-4 |
| | PND5 weight |
| | Pup neurobehavior, PND5 |
| | PND5-20 survival |
| | PND5-20 weight gain |
| | Body weight, PND20 |
| | Body weight, PND60, 90, 120 |
| | Body weight gain, PND20-60, 20-90, 20-120 |
| | Immune function |
| | Survival to PND 200 |
| | Post-necropsy tissue weights |
| | Hematology |
| | Serum chemistries |
| | Sperm concentration and motility |

| Table 2: P1 experimental groups | | | |
|---|---|---|---|
| Group 1 | Negative Controls - Sham Implantation Surgery | | |
| Group 2 | Males: 12 Ta steel pellets | X | Females: 12 Ta steel pellets |
| Group 3 | Males: 12 DU pellets | X | Females: 12 Ta steel pellets |
| Group 4 | Males: 12 DU pellets | X | Females: 4 DU, 8 Ta steel pellets |
| Group 5 | Males: 12 DU pellets | X | Females: 8 DU, 4 Ta steel pellets |
| Group 6 | Males: 12 DU pellets | X | Females: 12 DU pellets |
| Group 7 | Males: 12 Ta steel pellets | X | Females: 4 DU, 8 Ta steel pellets |
| Group 8 | Males: 12 Ta steel pellets | X | Females: 8 DU, 4 Ta steel pellets |
| Group 9 | Males: 12 Ta steel pellets | X | Females: 12 DU pellets |
| Group 10 | Males: 20 DU pellets | X | Females: 20 Ta steel pellets |
| Group 11 | Males: 20 Ta steel pellets | X | Females: 20 DU pellets |
| Group 12 | Males: 20 DU pellets | X | Females: 20 DU pellets |
| Group 13 | Males: 20 Ta steel pellets | X | Females: 20 Ta steel pellets |

| Table 3: Schedule of tone presentation in the 8 blocks of a single acoustic startle/prepulse inhibition session. | |
|---|---|
| Block 1 | 10 presentations of 105dB startle tone |
| Block 2 | 10 presentations of 74dB pre-pulse tone |
| Block 3 | 10 presentations of 74dB pre-pulse followed by 105dB startle tones |
| Block 4 | 10 presentations of 105dB startle tone |
| Block 5 | 10 presentations of 74dB pre-pulse followed by 105dB startle tones |
| Block 6 | 10 presentations of 105dB startle tone |
| Block 7 | 10 presentations of 74dB pre-pulse followed by 105dB startle tones |
| Block 8 | 10 presentations of 105dB startle tone |

Table 4: Toxicological endpoints measured in adult male rats at 120 and 150 days post-implantation surgery (± 95% Confidence Interval). n=(x).

| Endpoint | Sham surgery | 12 Ta steel pellets | 12 DU pellets | 20 Ta steel pellets | 20 DU pellets |
|---|---|---|---|---|---|
| Mean body weight, 120 d pi (grams) | 613 ± 55 (15) | 615 ± 67 (42) | 578 ± 33 (40) | 574 ± 40 (17) | 567 ± 44 (17) |
| Mean body weight, 150 d pi (grams) | 619 ± 81 (11) | 620 ± 37 (43) | 603 ± 24 (43) | - | - |
| Mean weight gain, 0 - 120 d pi (grams) | 339 ± 80 (15) | 271 ± 35 (42) | 265 ± 29 (40) | 301± 31 (17) | 300 ± 37 (17) |
| Mean weight gain, 0 - 150 d pi (grams) | 287± 95 (11) | 297 ± 28 (43) | 289 ± 21 (43) | - | - |

Table 5: Toxicological endpoints measured in adult female rats at 120 and 150 days post-implantation surgery (± 95% Confidence Interval). n=(x).

| Endpoint | Sham surgery | 12 Ta steel pellets | 4 DU pellets | 8 DU pellets | 12 DU pellets | 20 Ta steel pellets | 20 DU pellets |
|---|---|---|---|---|---|---|---|
| Mean body weight, 120 d pi (grams) | 354.3 ± 27.2 (23) | 337.8 ± 19.7 (42) | 339.6 ± 19.7 (41) | 336.0 ± 21.2 (42) | 342.2 ± 19.1 (41) | 347.5 ± 22.0 (16) | 342.4 ± 34.6 (28) |
| Mean body weight, 150 d pi (grams) | 379.0 ± 38.3 (14) | 353.0 ± 13.1 (31) | 379.9 ± 22.5 (31) | 366.8 ± 16.2 (26) | 380.3 ± 21.9 (26) | - | - |
| Mean weight gain, 0 - 120 d pi (grams) | 139.9 ± 39.7 (16) | 132.2 ± 19.9 (42) | 131.6 ± 20.5 (41) | 125.3 ± 22.9 (41) | 129.2 ± 19.9 (41) | **176.7 ± 22.3 (17)[1]** | **182.6 ± 32.3 (29)[1]** |
| Mean weight gain, 0 - 150 d pi (grams) | 168.5 ± 36.3 (14) | 148.9 ± 12.1 (31) | 168.8 ± 21.4 (31) | 152.5 ± 14.3 (26) | 165.2 ± 21.0 (25) | - | - |

[1]Significantly different from sham surgery controls ($p \leq 0.05$)

Table 6: Serum chemistries measured in adult male rats following necropsy at 200 days post-implantation (± 95% Confidence Interval). n=(x). Standard reference ranges for the rat are indicated in column A.

| Endpoint | Sham surgery | 12 Ta steel pellets | 12 DU pellets | 20 Ta steel pellets | 20 DU pellets |
|---|---|---|---|---|---|
| Total Protein (5.3 – 6.9 g/dl) | 7 ± 0.2 (10) | 6 ± 0.2 (7) | 7 ± 0.2 (10) | - | 6 ± 0.5 (5) |
| ALKP (16 – 302 U/L) | 10 ± 0 (10) | 11 ± 2 (7) | 13 ± 5 (10) | - | 10 ± 0 (5) |
| ALT (20 - 61 U/L) | 50 ± 10 (10) | 41 ± 4 (7) | 44 ± 5 (10) | - | 50 ± 14 (5) |
| AST (39 – 111 U/L) | 97 ± 26 (10) | 73 ± 22 (7) | 58 ± 8 (10) | - | 96 ± 16 (5) |
| PHOS (5.8 – 11.2 mg/dl) | 6 ± 0.1 (10) | 7 ± 1 (7) | 8 ± 1 (10) | - | 5 ± 1 (5) |
| CREA (0.05 – 0.65 mg/dl) | 0.3 ± 0.1 (10) | 0.4 ± 0.1 (7) | 0.4 ± 0.1 (10) | - | 0.3 ± 0.1 (5) |
| TBIL (0.1 – 0.7 mg/dl) | 0.3 ± 0.1 (10) | 0.2 ± 0.2 (7) | 0.3 ± 0.2 (10) | - | 0.3 ± 0.2 (5) |
| BUN (9 - 21 mg/dl) | 17 ± 2 (10) | 18 ± 2 (7) | 18 ± 2 (10) | - | 17 ± 5 (5) |

Table 7: Serum chemistries measured in adult female rats following necropsy at 200 days post-implantation (± 95% Confidence Interval). n=(x). Standard reference ranges for the rat are indicated in column A.

| Endpoint | Sham surgery | 12 Ta steel pellets | 4 DU pellets | 8 DU pellets | 12 DU pellets | 20 Ta steel pellets | 20 DU pellets |
|---|---|---|---|---|---|---|---|
| Total Protein (5.3 – 6.9 g/dl) | 6.7 ± 0.1 (10) | 7.1 ± 0.4 (10) | 6.9 ± 0.4 (10) | 7.0 ± 0.3 (10) | 7.1 ± 0.2 (11) | - | 6.5 ± 0.2 (5) |
| ALKP (16 – 302 U/L) | 12.6 ± 2.8 (10) | 12.1 ± 2.5 (10) | 11.6 ± 1.6 (10) | 13.2 ± 2.9 (10) | 13.1 ± 2.6 (11) | - | 16.4 ± 7.2 (5) |
| ALT (20 – 61 U/L) | 81.9 ± 43.8 (10) | **46.4 ± 11.9 (10)** | 59.8 ± 18.5 (10) | 51.6 ± 6.4 (10) | 76.5 ± 36.7 (11) | - | **44.8 ± 20.0 (5)**[1] |
| AST (39 – 111 U/L) | 106.0 ± 76.1 (10) | 76.5 ± 35.0 (10) | 85.2 ± 39.8 (10) | 78.8 ± 36.3 (10) | 119.7 ± 71.1 (11) | - | 52.8 ± 17.9 (5) |
| PHOS (5.8 – 11.2 mg/dl) | 6.7 ± 0.9 (10) | 6.8 ± 0.6 (10) | 7.3 ± 1.0 (10) | 7.3 ± 0.6 (10) | 6.9 ± 0.9 (11) | - | **5.0 ± 0.8 (5)**[1] |
| CREA (0.05 – 0.65 mg/dl) | 0.4 ± 0.0 (10) | 0.4 ± 0.0 (10) | 0.4 ± 0.1 (10) | 0.4 ± 0.0 (10) | 0.4 ± 0.0 (11) | - | 0.4 ± 0.1 (5) |
| TBIL (0.1 – 0.7 mg/dl) | 0.2 ± 0.2 (10) | 0.2 ± 0.1 (10) | 0.2 ± 0.1 (10) | 0.2 ± 0.1 (10) | 0.2 ± 0.1 (11) | - | 0.2 ± 0.3 (5) |
| BUN (9 - 21 mg/dl) | 17.0 ± 1.4 (10) | 18.4 ± 2.0 (10) | 17.2 ± 2.1 (10) | 20.4 ± 2.8 (10) | 18.5 ± 1.6 (11) | - | 16.7 ± 2.9 (5) |

[1]Significantly different from sham surgery controls ($p \leq 0.05$)

Table 8: Hematology values measured in adult P1 rats following necropsy at 200 days post-implantation (± 95% Confidence Interval n=(x).

| Endpoint | Sham surgery | 12 Ta steel pellets | 12 DU pellets | 20 Ta steel pellets | 20 DU pellets |
|---|---|---|---|---|---|
| WBC ($10^3$ µl) | 13.9 ± 4.7 (31) | 8.4 ± 1.1 (48) | 10.1 ± 2.2 (64) | 12.8 ± 5.8 (28) | 7.5 ± 0.9 (44) |
| % Lymphocytes | 84.6 ± 3.1 (26) | 85.6 ± 2.4 (44) | 87.6 ± 1.6 (61) | 78.8 ± 3.8 (28) | 82.4 ± 1.3 (42) |
| % Monocytes | 7.1 ± 1.2 (26) | 7.9 ± 1.1 (44) | 6.9 ± 0.8 (61) | 9.0 ± 1.2 (28) | **9.4 ± 0.9 (42)**[1] |
| % Granulocytes | 8.4 ± 2.7 (26) | 6.5 ± 1.6 (44) | 5.5 ± 1.1 (61) | 12.2 ± 3.7 (28) | 8.2 ± 0.9 (42) |
| # Lymphocytes | 8.2 ± 2.3 (26) | 7.7 ± 0.7 (44) | 7.5 ± 0.5 (60) | 9.2 ± 3.4 (28) | 5.9 ± 0.6 (42) |
| # Monocytes | 0.6 ± 0.1 (26) | 0.7 ± 0.1 (44) | 0.6 ± 0.1 (60) | 1.0 ± 0.3 (28) | 0.7 ± 0.1 (42) |
| # Granulocytes | 1.2 ± 1.3 (26) | 0.6 ± 0.3 (44) | 0.5 ± 0.1 (55) | 2.6 ± 2.3 (28) | 0.6 ± 0.1 (42) |
| RBC ($10^6$ µl) | 7.4 ± 0.2 (18) | 6.5 ± 1.0 (26) | 7.3 ± 0.4 (37) | 7.6 ± 0.1 (17) | 7.5 ± 0.2 (30) |
| HgB (g/dl) | 15.1 ± 0.4 (31) | 14.1 ± 1.0 (55) | 15.4 ± 1.1 (66) | 15.6 ± 0.3 (28) | 15.2 ± 0.3 (45) |
| Hematocrit % | 43.2 ± 1.1 (19) | 37.9 ± 5.7 (26) | 42.9 ± 2.5 (37) | 43.7 ± 1.0 (17) | 43.2 ± 1.1 (30) |
| MCV $mm^3$ | 57.0 ± 1.3 (30) | 56.1 ± 1.1 (50) | 57.1 ± 1.0 (65) | 56.3 ± 1.3 (26) | 56.3 ± 1.0 (44) |
| MCH (pg) | 19.8 ± 0.5 (18) | 20.0 ± 0.8 (26) | 19.9 ± 0.4 (37) | 20.1 ± 0.3 (17) | 19.8 ± 0.3 (30) |
| MCHC (g/dl) | 33.9 ± 0.8 (18) | 34.9 ± 1.6 (26) | 34.0 ± 0.6 (37) | 35.1 ± 0.9 (17) | 34.5 ± 0.6 (30) |

Table 8: Hematology values measured in adult P1 rats following necropsy at 200 days post-implantation (± 95% Confidence Interval n=(x).

| Endpoint | Sham surgery | 12 Ta steel pellets | 12 DU pellets | 20 Ta steel pellets | 20 DU pellets |
|---|---|---|---|---|---|
| RDW% | 14.9 ± 1.5 (31) | 14.9 ± 1.0 (50) | 14.2 ± 0.6 (65) | 14.0 ± 1.0 (27) | 13.3 ± 0.6 (45) |
| PLT ($10^3$ µl) | 1038.0 ± 69.6 (31) | 942.0 ± 82.3 (51) | 1013.8 ± 52.2 (65) | 964.6 ± 95.1 (28) | **890.3 ± 54.8 (45)**[1] |
| MPV (fl) | 8.1 ± 0.3 (31) | 8.0 ± 0.3 (50) | 8.1 ± 0.2 (65) | 7.9 ± 0.3 (28) | 7.8 ± 0.2 (45) |

[1]Significantly different from sham surgery controls ($p \leq 0.05$)

| Table 9: Mean P1 male organ weights in grams (± 95% confidence interval). n=(x). | | | | | |
|---|---|---|---|---|---|
| Organ or organ system | Sham surgery | 12 Ta steel pellets | 12 DU pellets | 20 Ta steel pellets | 20 DU pellets |
| Sex organs | 12.9 ± 0.4 (12) | **10.6 ± 1.3 (28)[1]** | **10.9 ± 0.9 (39)[1]** | 11.4 ± 0.9 (10) | 11.7 ± 1.3 (13) |
| Liver | 21.7 ± 1.9 (24) | 21.2 ± 0.2 (46) | 21.8 ± 0.8 (65) | 20.2 ± 1.9 (13) | 20.4 ± 1.8 (18) |
| Kidneys | 4.9 ± 0.4 (23) | 4.5 ± 0.2 (47) | 4.5 ± 0.2 (66) | 5.0 ± 0.7 (13) | 5.1 ± 0.4 (18) |
| Heart | 2.0 ± 0.1 (18) | 2.2 ± 0.2 (21) | 2.0 ± 0.1 (39) | 2.2 ± 0.3 (12) | 2.2 ± 0.2 (17) |
| Spleen | 0.9 ± 0.2 (19) | 1.1 ± 0.2 (21) | 1.0 ± 0.1 (40) | 1.0 ± 0.1 (13) | 1.1 ± 0.1 (18) |
| Brain | 2.0 ± 0.1 (23) | 2.1 ± 0.1 (48) | 2.0 ± 0.1 (66) | 2.0 ± 0.2 (13) | 2.1 ± 0.1 (18) |
| [1]Significantly different from sham surgery controls ($p \leq 0.05$) | | | | | |

| Table 10: Mean P1 female organ weights in grams (± 95% confidence interval). n=(x). | | | | | | | |
|---|---|---|---|---|---|---|---|
| Organ or organ system | Sham Surgery | 12 Ta steel pellets | 4 DU, 8 Ta Steel | 8 DU, 4 Ta Steel | 12 DU | 20 Ta Steel | 20 DU |
| Sex Organs | 1.2 ± 0.2 (4) | 1.1 ± 0.1 (27) | 1.2 ± 0.1 (26) | 1.3 ± 0.2 (28) | 1.2 ± 0.2 (28) | 1.2 ± 0.1 (28) | 1.2 ± 0.2 (15) |
| Liver | 13.2 ± 0.8 (21) | 13.9 ± 1.0 (27) | 13.2 ± 0.8 (28) | 14.8 ± 1.0 (28) | 13.6 ± 0.6 (29) | 14.3 ± 1.2 (27) | 14.0 ± 1.3 (14) |
| Kidneys | 2.9 ± 0.2 (20) | 2.9 ± 0.1 (27) | 2.9 ± 0.1 (28) | 3.1 ± 0.2 (27) | 3.0 ± 0.2 (29) | 3.3 ± 0.2 (27) | 3.2 ± 0.2 (15) |
| Heart | 1.5 ± 0.1 (17) | 1.5 ± 0.1 (16) | 1.6 ± 0.2 (17) | 1.7 ± 0.2 (15) | 1.6 ± 0.2 (18) | 1.8 ± 0.1 (28) | 1.8 ± 0.1 (15) |
| Spleen | 0.6 ± 0.1 (17) | 0.6 ± 0.1 (16) | 0.7 ± 0.1 (17) | 0.8 ± 0.2 (15) | 0.7 ± 0.1 (16) | 0.7 ± 0.1 (28) | 0.7 ± 0.1 (14) |
| Brain | 1.8 ± 0.1 (21) | 1.7 ± 0.1 (26) | 1.7 ±0.1 (28) | 1.9 ± 0.1 (29) | 1.7 ± 0.1 (29) | 1.9 ± 0.1 (28) | 1.8 ± 0.2 (15) |

Table 11: Mean normalized P1 male organ weights in grams (± 95% confidence interval). n=(x). All values are $x10^{-3}$.

| Organ or organ system | Sham surgery | 12 Ta steel pellets | 12 DU pellets | 20 Ta steel pellets | 20 DU pellets |
|---|---|---|---|---|---|
| Sex organs | 21.6 ± 3.0 (12) | 21.8 ± 3.1 (15) | 21.7 ± 2.2 (27) | 19.4 ± 1.1 (10) | 21.0 ± 4.8 (12) |
| Liver | 36.5 ± 3.5 (15) | 39.7 ± 5.9 (17) | 40.1 ± 3.6 (31) | 34.9 ± 2.2 (13) | 37.4 ± 6.6 (17) |
| Kidneys | 8.4 ± 0.9 (15) | 8.4 ± 1.0 (17) | 8.6 ± 0.8 (31) | 8.6 ± 1.0 (13) | 9.0 ± 1.1 (17) |
| Heart | 3.4 ± 0.4 (15) | 3.9 ± 0.7 (18) | 3.9 ± 0.5 (30) | 3.9 ± 0.4 (12) | 4.0 ± 0.7 (16) |
| Spleen | 1.6 ± 0.3 (15) | 1.8 ± 0.3 (18) | 1.8 ± 0.3 (31) | 1.7 ± 0.2 (13) | 1.9 ± 0.3 (17) |
| Brain | 3.5 ± 0.5 (14) | 3.6 ± 0.6 (18) | 3.7 ± 0.4 (31) | 3.5 ± 0.4 (13) | 3.8 ± 0.5 (17) |

Table 12: Mean normalized P1 female organ weights in grams (± 95% confidence interval). n=(x). All values are $x10^{-3}$.

| Organ or organ system | Sham Surgery | 12 Ta steel pellets | 4 DU, 8 Ta steel pellets | 8 DU, 4 Ta steel pellets | 12 DU pellets | 20 Ta steel pellets | 20 DU pellets |
|---|---|---|---|---|---|---|---|
| Sex Organs | 3.2 ± 0.5 (18) | 3.3 ± 0.5 (27) | 3.5 ± 0.5 (26) | 3.8 ± 0.5 (27) | 3.6 ± 0.5 (28) | 3.4 ± 0.4 (27) | 3.5 ± 0.6 (15) |
| Liver | 38.1 ± 2.4 (20) | 38.9 ± 2.6 (25) | 40.4 ± 2.7 (26) | 43.0 ± 2.5 (24) | 40.2 ± 2.2 (28) | 40.9 ± 2.8 (24) | 40.5 ± 3.8 (14) |
| Kidneys | 8.6 ± 0.5 (19) | 8.5 ± 0.5 (26) | 8.8 ± 0.4 (26) | 8.9 ± 0.5 (25) | 8.9 ± 0.6 (26) | 9.3 ± 0.5 (24) | 9.3 ± 0.6 (15) |
| Heart | 4.3 ± 0.3 (17) | 4.4 ± 0.4 (16) | 4.5 ± 0.5 (17) | 4.7 ± 0.6 (15) | 4.6 ± 0.5 (18) | **5.2 ± 0.3 (25)[1]** | 5.1 ± 0.5 (15) |
| Spleen | 1.7 ± 0.2 (17) | 1.6 ± 0.3 (16) | 1.9 ± 0.3 (17) | 2.0 ± 0.4 (14) | 1.8 ± 0.3 (16) | 2.0 ± 0.2 (26) | 2.1 ± 0.3 (14) |
| Brain | 5.3 ± 0.4 (20) | 5.5 ± 0.3 (24) | 5.5 ± 0.4 (26) | 5.7 ± 0.3 (26) | 5.3 ± 0.3 (27) | 5.6 ± 0.4 (26) | 5.3 ± 0.7 (14) |

[1]Significantly different from sham surgery controls ($p \leq 0.05$)

| Table 13: Histopathologic lesions identified in P1 adult animals euthanized and that underwent necropsy at 200 days post-implantation surgery | | | |
|---|---|---|---|
| Tissue examined and identified lesion | Treatment group and number of animals with at least one of the indicated lesions | | |
| | Sham surgery controls (N=10) | 20 Ta pellets (N=13) | 20 DU pellets (N=14) |
| **Lung** | | | |
| Inflammation, granuloma | 3 | 1 | 0 |
| Inflammation, perivascular, mononuclear | 2 | 4 | 1 |
| Histiocytosis, alveolar | 2 | 2 | 4 |
| Hemorrhage, acute | 2 | 6 | 3 |
| Mineralization, vascular | 1 | 1 | 0 |
| **Spleen** | | | |
| Hemosiderosis | 2 | 1 | 2 |
| Congestion | 0 | 0 | 0 |
| **Thymus** | | | |
| Hemorrhage, acute | 0 | 1 | 4 |
| **Kidney** | | | |
| Inflammation, interstial | 0 | 4 | 0 |
| Mineralization | 1 | 2 | 2 |
| Nephropathy | 1 | 2 | 2 |
| **Liver** | | | |
| Inflammation, mixed, random | 8 | 5 | 7 |
| Granulomatous arteritis | 0 | 1 | 0 |
| Portal inflammation | 3 | 3 | 2 |
| **Uterus** | | | |
| Hemosiderosis | 1 | 3 | 1 |
| Amyloidosis | 0 | 0 | 1 |
| **Ovary** | None | None | None |
| **Testes** | None | None | None |
| **Bone marrow** | None | None | None |

Table 14: Sperm motion parameters for P1 males undergoing euthanasia and necropsy at 200 days post-implantation surgery

| Treatment group | N | % motile | % progressive | VAP[a] (μm/sec) | VSL[b] (μm/sec) | VCL[c] (μm/sec) | ALH[d] (μm) |
|---|---|---|---|---|---|---|---|
| Sham surgery | 14 | 69 ± 7 | 22 ± 4 | 184 ± 12 | 128 ± 9 | 351 ± 15 | 15 ± 1 |
| α-chlorohydrin | 20 | 60 ± 4[e] | 20 ± 1 | 137 ± 5[f] | 95 ± 4[f] | 211 ± 7[f] | 10 ± 1[f] |
| 12 Ta steel pellets | 21 | 74 ± 4 | 21 ± 2 | 190 ± 8 | 130 ± 6 | 352 ± 13 | 15 ± 1 |
| 12 DU pellets | 35 | 74 ± 4 | 21 ± 2 | 186 ± 8 | 126 ± 6 | 355 ± 12 | 16 ± 1 |
| 20 Ta steel pellets | 16 | 73 ± 7 | 23 ± 4 | 196 ± 15 | 135 ± 11 | 380 ± 22[f] | 16 ± 1 |
| 20 DU pellets | 16 | 73 ± 5 | 24 ± 2 | 203 ± 15 | 141 ± 11 | 384 ± 25[f] | 16 ± 1 |

[a]VAP= Average path velocity; [b]VSL= Progressive straight line velocity; [c]VCL= Tract speed velocity; [d]ALH= Lateral sperm head displacement;
[e] $P \leq 0.05$ in Dunnett two-sided test versus sham surgery
[f] $P \leq 0.001$ in Dunnett two-sided test versus sham surgery

Table 15: P1 sperm concentrations for P1 males undergoing euthanasia and necropsy at 200 days post-implantation surgery

| Treatment group | N | # of Cells | Concentration ($10^6$ cells/mL) | Concentration ($10^6$ cells/gram tissue) |
|---|---|---|---|---|
| Sham surgery | 14 | 171 ± 50.7 | 19.8 ± 5.9 | 861.9 ± 262.0 |
| 12 Ta steel pellets | 18 | 177.6 ± 45.3 | 20.7 ± 5.4 | 959.1 ± 249.7 |
| 12 DU pellets | 26 | 186.2 ± 89.3 | 16.7 ± 3.5 | 674.3 ± 153.3 |
| 20 Ta steel pellets | 15 | 159.4 ± 46.0 | 18.4 ± 5.3 | 584.3 ± 154.0 |
| 20 DU pellets | 17 | 164.6 ± 37.6 | 19.1 ± 4.3 | 684.0 ± 194.6 |

Table 16: P1 female acoustic startle response magnitude at 150 days post-implantation surgery

| Treatment group | Number of animals tested | Response magnitude (SDI units)[1] | 95% CI |
|---|---|---|---|
| Sham surgery controls | 120 | 120.1 | 34.1 |
| 12 Ta steel pellets | 120 | 165.5 | 45.6 |
| 4 DU pellets | 120 | 77.2 | 20.2 |
| 12 DU pellets | 120 | 49.0 | 9.2 |
| 20 DU pellets | 120 | 92.2 | 22.4 |
| 20 Ta steel pellets | 120 | 97.4 | 25.5 |

[1]San Diego Instrument startle response system units representing 1.22 millivolts – 5 volts divided by 4,095.

Table 17: P1 male open field vertical plane movements when tested at 150 days post-implantation surgery

| Treatment group | Number of animals tested | Mean number of vertical plane movements | 95% CI |
|---|---|---|---|
| Sham surgery controls | 10 | 4.4 | 4.5 |
| 12 Ta steel pellets | 10 | **27.4**[1] | **3.7** |
| 12 DU pellets | 10 | **23.6**[1] | **4.4** |
| 20 DU pellets | 10 | **16.7**[1] | **5.8** |
| 20 Ta steel pellets | 10 | **22.6**[1] | **8.2** |

[1]Significantly different from sham surgery controls ($p \leq 0.05$)

| Table 18: P1 female coincidence contact frequency when tested at 150 days post-implantation surgery | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Response magnitude (SDI units)[1] | 95% CI |
| Sham surgery controls | 30 | 62.3 | 15.3 |
| 12 Ta steel pellets | 30 | 80.1 | 9.3 |
| 4 DU pellets | 30 | 85.9 | 10.6 |
| 12 DU pellets | 30 | 66.3 | 7.2 |
| 20 DU pellets | 30 | 52.9 | 11.2 |
| 20 Ta steel pellets | 30 | 96.8 | 30.6 |

Table 19: Reproductive success of P1 males mated at 30-45 days post-implantation surgery

| Treatment group | Mating success | Failure | Percent Mating Success | Average insemination time (days)* | 95% CI |
|---|---|---|---|---|---|
| Sham surgery | 17 | 4 | 81 | 1.9 | 0.5 |
| 12 Ta steel pellets | 65 | 7 | 90 | 2.2 | 0.5 |
| 12 DU pellets | 67 | 5 | 93 | 1.9 | 0.2 |
| 20 Ta steel pellets | 20 | 2 | 90 | 1.9 | 0.5 |
| 20 DU pellets | 22 | 3 | 88 | 2.0 | 0.5 |

*Number of days between introduction to female and appearance of vaginal plug in cage

Table 20: Gestation length and gestation weight gain of P1 females mated at 30-45 days post-implantation day surgery

| Treatment group | N | Gestation length (days) | Gestation weight gain (grams) |
|---|---|---|---|
| Sham surgery controls | 18 | 22.1 ± 0.3 | 123.1 ± 15.2 |
| 12 Ta steel pellets | 29 | 21.8 ± 0.2 | 128.3 ± 12.0 |
| 4 DU pellets | 29 | 21.8 ± 0.4 | 134.7 ± 7.3 |
| 8 DU pellets | 34 | 21.7 ± 0.4 | 128.2 ± 10.2 |
| 12 DU pellets | 33 | 21.9 ± 0.2 | 132.8 ± 7.1 |
| 20 Ta steel pellets | 8 | 21.6 ± 0.4 | 152.5 ± 24.2 |
| 20 DU pellets | 14 | 21.7 ± 0.5 | 135.2 ± 17.2 |

Table 21: Litter size, percentage of males per litter, and pup survival for litters born to mothers mated at 30-45 days post-implantation day surgery (± 95% Confidence Interval)

| Mating Group (from Table 2) | Number of litters | Litter size (PND1) | Percentage males | Percentage of pups surviving PND1-4 | Percentage of pups surviving PND5-20 |
|---|---|---|---|---|---|
| 1 | 17 | 14.7 ± 1.2 | 46.9 ± 6.2 | 92.2 ± 13.2 | 94.9 ± 6.8 |
| 2 | 15 | 15.3 ± 1.1 | 47.8 ± 7.9 | 94.9 ± 4.9 | 99.1 ± 1.9 |
| 3 | 16 | 14.3 ± 1.7 | 62.3 ± 4.8 | 93.2 ± 13.3 | 97.7 ± 2.7 |
| 4 | 15 | 14.9 ± 1.8 | 48.4 ± 8.9 | 96.2 ± 4.4 | 95.3 ± 7.2 |
| 5 | 16 | 14.5 ± 1.6 | 48.9 ± 7.9 | 98.0 ± 1.9 | 100.0 ± 0.0 |
| 6 | 18 | 15.2 ± 1.2 | 53.8 ± 8.3 | 93.3 ± 8.7 | 94.9 ± 6.5 |
| 7 | 14 | 14.8 ± 1.1 | 53.6 ± 7.7 | 99.2 ± 1.2 | 95.5 ± 7.8 |
| 8 | 18 | 14.8 ± 1.2 | 56.5 ± 6.0 | 98.5 ± 1.8 | 99.3 ± 1.5 |
| 9 | 17 | 14.3 ± 1.2 | 55.1 ± 6.6 | 98.3 ± 5.7 | 96.1 ± 5.3 |
| 10 | 14 | 15.8 ± 1.3 | 44.3 ± 6.8 | 92.0 ± 15.3 | 100.0 ± 0.0 |
| 11 | 7 | 14.4 ± 2.3 | 50.9 ± 17.0 | 97.0 ± 5.1 | 98.4 ± 3.7 |
| 12 | 6 | 17.8 ± 1.8 | 50.4 ± 10.4 | 97.6 ± 4.1 | 100.0 ± 0.0 |
| 13 | 11 | 14.5 ± 1.0 | 45.9 ± 7.3 | 90.0 ± 22.6 | 100.0 ± 0.0 |

Table 22: P1 mother average time to find pup and retrieve to nest (maternal retrieval) on F1a PND2 for pups born to mothers mated at 30-45 days post-implantation day surgery (± 95% Confidence Interval)

| Treatment group | Number of animals tested | Mean (sec) | 95% CI |
|---|---|---|---|
| 1 | 8 | 96.0 | 38.8 |
| 2 | 8 | 53.8 | 17.3 |
| 3 | 8 | 82.8 | 50.8 |
| 4 | 8 | 85.5 | 36.1 |
| 5 | 8 | 109.5 | 53.1 |
| 6 | 8 | 100.6 | 34.8 |
| 7 | 8 | 98.5 | 48.0 |
| 8 | 8 | 90.5 | 55.7 |
| 9 | 8 | 98.6 | 45.5 |
| 10 | 8 | **45.5**[1] | **7.5** |
| 11 | 6 | 122.3 | 22.1 |
| 12 | 4 | 78.4 | 30.4 |
| 13 | 5 | **55.2**[1] | **7.7** |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

Table 23: F1a pup average time to righting when assessed on PND4 (± 95% Confidence Interval)

| Parental treatment group | Number of animals tested | Mean (sec) | 95% CI |
|---|---|---|---|
| 1 | 16 | 14.0 | 2.7 |
| 2 | 16 | 10.5 | 2.1 |
| 3 | 16 | 9.1 | 1.4 |
| 4 | 16 | 9.1 | 4.0 |
| 5 | 16 | 8.7 | 3.5 |
| 6 | 16 | 11.9 | 2.6 |
| 7 | 16 | 9.6 | 3.2 |
| 8 | 16 | 11.8 | 3.1 |
| 9 | 16 | 8.5 | 1.9 |
| 10 | 19 | 9.2 | 2.2 |
| 11 | 6 | 7.9 | 2.5 |
| 12 | 7 | 11.9 | 3.2 |
| 13 | 10 | 12.1 | 6.1 |

Table 24: F1a pup vocalization frequency in response to separation from its P1 mother on PND7 (± 95% Confidence Interval)

| Treatment group | Number of animals tested | Mean (vocs/min) | 95% CI |
|---|---|---|---|
| 1 | 16 | 27.0 | 6.9 |
| 2 | 16 | 28.5 | 5.3 |
| 3 | 16 | 30.6 | 6.7 |
| 4 | 16 | 17.5 | 2.4 |
| 5 | 16 | 13.4 | 2.2 |
| 6 | 16 | 18.8 | 4.5 |
| 7 | 16 | 16.9 | 4.5 |
| 8 | 16 | 14.3 | 1.8 |
| 9 | 16 | 14.4 | 3.0 |
| 10 | 56 | 30.9 | 6.8 |
| 11 | 24 | 32.9 | 11.8 |
| 12 | 32 | 31.6 | 10.8 |
| 13 | 48 | 34.6 | 9.3 |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

| Table 25: F1a PND17-32 juvenile play test results: dorsal contacts (± 95% Confidence Interval) | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of contacts) | 95% CI |
| 1 | 96 | 31.7 | 15.2 |
| 2 | 96 | 28.5 | 3.6 |
| 4 | 96 | 25.9 | 2.7 |
| 6 | 96 | 25.9 | 2.9 |
| 10 | 60 | 27.3 | 2.3 |
| 11 | 36 | 21.9 | 4.6 |
| 12 | 84 | 26.1 | 2.7 |
| 13 | 36 | 26.9 | 5.9 |

Table 26: F1a PND17-32 juvenile play test results: pins (± 95% Confidence Interval)

| Treatment group | Number of animals tested | Mean (# of pins) | 95% CI |
|---|---|---|---|
| 1 | 96 | 10.5 | 1.9 |
| 2 | 96 | 7.6 | 1.7 |
| 4 | 96 | 9.7 | 1.7 |
| 6 | 96 | 8.1 | 1.7 |
| 10 | 60 | 7.8 | 11.9 |
| 11 | 36 | 5.8 | 13.0 |
| 12 | 84 | 6.9 | 11.0 |
| 13 | 36 | 8.4 | 13.5 |

| Table 27: F1a PND17-32 juvenile play test results: pin latency in seconds (± 95% Confidence Interval) | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean pin latency (seconds) | 95% CI |
| 1 | 96 | 1.7 | 0.2 |
| 2 | 96 | 1.5 | 0.2 |
| 4 | 96 | 1.5 | 0.2 |
| 6 | 96 | 1.5 | 0.3 |
| 10 | 60 | 11.2 | 2.6 |
| 11 | 36 | 8.9 | 3.8 |
| 12 | 84 | 12.1 | 2.6 |
| 13 | 36 | 16.5 | 5.6 |

| Table 28: F1a Spontaneous Locomotor Activity assessment at PND32: distance traveled | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean distance traveled (cm) | 95% CI |
| 1 | 22 | 1177.0 | 105.9 |
| 2 | 22 | 928.7 | 147.0 |
| 3 | 22 | 968.8 | 238.2 |
| 4 | 22 | 883.5 | 198.7 |
| 5 | 22 | 959.4 | 204.0 |
| 6 | 22 | 1211.7 | 218.7 |
| 7 | 22 | 941.2 | 211.5 |
| 8 | 22 | 1057.2 | 168.8 |
| 9 | 22 | 1131.6 | 97.0 |
| 10 | 24 | 1128.529 | 65.2 |
| 11 | 24 | 990.988 | 136.6 |
| 12 | 24 | 1133.892 | 78.8 |
| 13 | 24 | **1188.896**[1] | **83.3** |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 29: F1a Spontaneous Locomotor Activity assessment at PND32: time resting | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean time resting (seconds) | 95% CI |
| 1 | 22 | 98.7 | 9.6 |
| 2 | 22 | 125.5 | 24.6 |
| 3 | 22 | 138.3 | 32.9 |
| 4 | 22 | 133.5 | 34.0 |
| 5 | 22 | 135.6 | 33.9 |
| 6 | 22 | 106.4 | 26.6 |
| 7 | 22 | 134.5 | 34.0 |
| 8 | 22 | 113.8 | 25.8 |
| 9 | 22 | 95.3 | 6.4 |
| 10 | 24 | **91.5**[1] | **6.3** |
| 11 | 24 | 113.1 | 13.2 |
| 12 | 24 | 104.9 | 4.7 |
| 13 | 24 | **94.6**[1] | **5.9** |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 30: F1a Spontaneous Locomotor Activity assessment at PND32: stereotypical behavior count | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 22 | 29.1 | 15.0 |
| 2 | 22 | 17.3 | 2.7 |
| 3 | 22 | 17.6 | 4.1 |
| 4 | 22 | 16.4 | 3.6 |
| 5 | 22 | 17.5 | 15.5 |
| 6 | 22 | 22.1 | 3.7 |
| 7 | 22 | 17.4 | 3.9 |
| 8 | 22 | 19.6 | 3.1 |
| 9 | 22 | 21.0 | 1.6 |
| 10 | 24 | **187.5**[1] | **5.6** |
| 11 | 24 | 168.5 | 11.0 |
| 12 | 24 | 174.2 | 4.1 |
| 13 | 24 | 183.4 | 4.9 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

Table 31: F1a Spontaneous Locomotor Activity assessment at PND32: time ambulatory

| Treatment group | Number of animals tested | Mean time ambulatory (seconds) | 95% CI |
|---|---|---|---|
| 1 | 22 | 172.2 | 9.7 |
| 2 | 22 | 157.2 | 22.0 |
| 3 | 22 | 144.1 | 29.2 |
| 4 | 22 | 150.1 | 30.6 |
| 5 | 22 | 146.8 | 30.3 |
| 6 | 22 | 171.5 | 23.7 |
| 7 | 22 | 148.0 | 30.4 |
| 8 | 22 | 166.6 | 23.1 |
| 9 | 22 | 183.7 | 5.5 |
| 10 | 24 | 21.0 | 1.2 |
| 11 | 24 | 18.4 | 2.5 |
| 12 | 24 | 21.0 | 1.4 |
| 13 | 24 | 22.0 | 1.5 |
| 13 | 24 | 18.4 | 2.5 |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

| Table 32: F1a Spontaneous Locomotor Activity assessment at PND32: stereotypical bursts | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 22 | 311.3 | 11.3 |
| 2 | 22 | 264.8 | 37.8 |
| 3 | 22 | 257.2 | 54.4 |
| 4 | 22 | 255.2 | 53.0 |
| 5 | 22 | 252.5 | 51.8 |
| 6 | 22 | 304.5 | 44.1 |
| 7 | 22 | 256.0 | 53.6 |
| 8 | 22 | 285.4 | 40.4 |
| 9 | 22 | 311.0 | 14.6 |
| 10 | 24 | 309.3 | 11.6 |
| 11 | 24 | 286.7 | 27.0 |
| 12 | 24 | 309.8 | 11.3 |
| 13 | 24 | **320.8**[1] | **12.2** |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 33: F1a Spontaneous Locomotor Activity assessment at PND32: horizontal movements | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 22 | 1338.3 | 150.7 |
| 2 | 22 | 1039.3 | 164.0 |
| 3 | 22 | 1057.5 | 227.2 |
| 4 | 22 | 1055.4 | 247.5 |
| 5 | 22 | 1251.5 | 310.8 |
| 6 | 22 | 1338.8 | 210.9 |
| 7 | 22 | 1083.3 | 229.3 |
| 8 | 22 | 1299.7 | 232.5 |
| 9 | 22 | 1379.9 | 120.1 |
| 10 | 24 | 1562.1 | 151.8 |
| 11 | 24 | 1293.1 | 154.8 |
| 12 | 24 | 1361.3 | 79.9 |
| 13 | 24 | **1509.0**[1] | **149.5** |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 34: F1a Spontaneous Locomotor Activity assessment at PND32: ambulatory movements | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 22 | 1003.3 | 140.2 |
| 2 | 22 | 769.0 | 127.4 |
| 3 | 22 | 790.0 | 177.3 |
| 4 | 22 | 799.5 | 200.2 |
| 5 | 22 | 927.1 | 209.9 |
| 6 | 22 | 1002.2 | 174.9 |
| 7 | 22 | 815.6 | 178.7 |
| 8 | 22 | 973.1 | 183.7 |
| 9 | 22 | 1027.7 | 102.1 |
| 10 | 24 | 1190.7 | 132.7 |
| 11 | 24 | 971.3 | 138.5 |
| 12 | 24 | 1043.9 | 67.5 |
| 13 | 24 | 1155.9 | 137.8 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

Table 35: F1a Spontaneous Locomotor Activity assessment at PND32: # of rears

| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
|---|---|---|---|
| 1 | 22 | 25.9 | 10.8 |
| 2 | 22 | 27.3 | 11.6 |
| 3 | 22 | 34.8 | 12.7 |
| 4 | 22 | 27.4 | 10.5 |
| 5 | 22 | 35.4 | 12.5 |
| 6 | 22 | 34.5 | 12.2 |
| 7 | 22 | 25.8 | 10.6 |
| 8 | 22 | 33.1 | 10.6 |
| 9 | 22 | 40.0 | 13.0 |
| 10 | 24 | 19.5 | 8.7 |
| 11 | 24 | 10.3 | 5.8 |
| 12 | 24 | 29.4 | 13.3 |
| 13 | 24 | 33.8 | 26.5 |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

| Table 36: F1a Spontaneous Locomotor Activity assessment at PND32: # vertical plane movements | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 22 | 11.6 | 5.5 |
| 2 | 22 | 11.3 | 4.6 |
| 3 | 22 | 14.1 | 5.8 |
| 4 | 22 | 11.7 | 4.9 |
| 5 | 22 | 16.6 | 5.7 |
| 6 | 22 | 15.6 | 5.6 |
| 7 | 22 | 11.6 | 4.8 |
| 8 | 22 | 14.7 | 5.3 |
| 9 | 22 | 16.5 | 5.3 |
| 10 | 24 | 9.8 | 4.4 |
| 11 | 24 | 5.9 | 3.1 |
| 12 | 24 | 13.1 | 4.5 |
| 13 | 24 | 11.6 | 4.5 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

Table 37: F1a acoustic startle response magnitude when assessed at PND45-47

| Treatment group | Number of animals tested | Response magnitude (SDI units)[1] | 95% CI |
|---|---|---|---|
| 1 | 288 | 85.4 | 11.4 |
| 2 | 288 | 90.2 | 16.1 |
| 6 | 288 | 75.7 | 10.5 |
| 10 | 108 | 125.9 | 13.4 |
| 11 | 108 | 115.2 | 15.7 |
| 12 | 108 | 125.2 | 12.4 |
| 13 | 108 | 123.2 | 12.5 |

[1]San Diego Instrument startle response system units representing 1.22 millivolts – 5 volts divided by 4,095

| Table 38: F1a acoustic startle response latency when assessed at PND45-47 | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Response latency (seconds) | 95% CI |
| 1 | 288 | 85.4 | 11.4 |
| 2 | 288 | 90.2 | 16.1 |
| 6 | 288 | 75.7 | 10.5 |
| 10 | 108 | 125.9 | 13.4 |
| 11 | 108 | 123.2 | 12.5 |
| 12 | 108 | 115.2 | 15.7 |
| 13 | 108 | 125.2 | 12.4 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 39: F1a time to find the platform in the Morris Watermaze test at PND59-63 | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Time to find platform (seconds) | 95% CI |
| 2 | 30 | 20.3 | 7.4 |
| 6 | 30 | 25.6 | 6.7 |
| 10 | 30 | 28.0 | 9.9 |
| 11 | 30 | 22.3 | 8.7 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

Table 40: F1a mean path distance to find the platform in the Morris Watermaze test at PND59-63

| Treatment group | Number of animals tested | Path distance to platform (cm) | 95% CI |
|---|---|---|---|
| 2 | 30 | 433.6 | 140.3 |
| 6 | 30 | 398.8 | 94.2 |
| 10 | 30 | 499.5 | 163.1 |
| 11 | 30 | 348.4 | 124.3 |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

Table 41: Hematology values measured in adult F1a rats following necropsy at PND90 (± 95% Confidence Interval). n=(x).

| Measurement | Parental treatment group | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| WBC $10^3$ µl | 13.5 ±2.2 (13) | 11.8 ± 2 (15) | 11.4 ± 1.4 (16) | 12.2 ± 2.4 (15) | 13.2 ± 1.7 (22) | 11.5 ± 1.4 (16))\ | 13.2 ± 1.9 (14) | 14.6 ± 1.6 (18) | 11.2 ± 2.3 (18) | **12.9 ± 2.6[2] (8)** | 12.48 ± 2.4 (5) |
| Lymphocytes % | 90.8 ± .89 (15) | 90.4 ± 1.6 (14) | 90.5 ± 1.5 (17) | 89.8 ± 1.3 (14) | 90.7 ± 1.2 (23) | 90.2 ± 1.4 (17) | 88.8 ± 2 (13) | 90.1 ± 1. (18) | 91.2 ± 1.5 (20) | 89.2 ± 2.3 8 | 90.8 ± 2.6 (6) |
| monocytes % | 6.4 ± 0.9 (15) | 7.0 ± 1.3 (14) | 5.6 ± .8 (17) | 6.8 ± .8 (14) | 6 ± .7 (23) | 6.4 ± .9 (17) | 6.4 ± 1.5 (13) | 6.6 ± .8 (18) | 5.8 ± 1 (20) | 6.8 ± 1.2 8 | 5.8 ± 1.4 6 |
| granulocytes $10^3$ µl | 2.8 ± .6 (15) | 2.6 ± .6 (14) | 3.9 ± 1.2 (17) | 3.4 ± .8 (14) | 3.2 ± .8 (23) | 3.4 ± .8 (17) | 4.8 ± .9 (13) | 3.2 ± .7 (18) | 3 ± .6 (20) | 4.1 ± 1.5 8 | 3.4 ± 1.7 6 |
| #lymphocytes | 12.3 ± 2.0 (13) | 11.3 ± 1.3 (14) | 10.5 ± 1.3 (15) | 11.7 ± 1.5 (14) | 11.9 ± 1.5 (22) | 10.3 ± 1.2 (16) | 12.2 ± 1.7 (13) | 13.2 ± 1.4 (18) | 10.1 ± 2.1 (18) | 11.5 ± 2.3 8 | 11.6 ± 2.4 6 |
| #monocytes | .9 ± .2 (13) | .9 ± .2 (14) | .7 ± .1 (16) | .9 ± .2 (14) | .8 ± .1 (22) | .8 ± .2 (16) | .8 ± .2 (13) | 1 ± .2 (18) | .7 ± .2 (18) | .9 ± .2 8 | .8 ± .3 6 |
| #granulocytes | .4 ± .1 (13) | .3 ± .1 (14) | .4 ± .1 (14) | .45 ± .1 (14) | .4 ± .1 (22) | .4 ± .1 (15) | .6 ± .1 (13) | .5 ± .1 (18) | .4 ± .1 (17) | .5 ± .2 8 | .4 ± .2 6 |
| RBC $10^6$ µl | 7.2 ± .3 (15) | 7.0 ± 1.7 (8) | 7. ± .9 (14) | 7 ± 1.2 (13) | 7.2 ± .4 (20) | 6.9 ± .9 (15) | 6.8 ± 1.1 (13) | 7.6 ± .3 (16) | 6.7 ± .8 (15) | 7.4 ± .2 8 | 7.5 ± .2 5 |
| HgB g/dl | 15.0 ± .6 (17) | 14.8 ± 2 (16) | 14.4 ± 1.4 (18) | 14.6 ± 1.9 (17) | 15.1 ± .7 (25) | 14.7 ± 1.5 (19) | 14.3 ± 1.9 (16) | 15.42 ± .7 (20) | 14.4 ± 1 (22) | 14.6 ± .5 8 | 14.8 ±. 7 6 |
| Hematocrit % | 44.9 ± 1.5 (15) | 42.2 ± 10.3 (8) | 42.3 ± 5.9 (14) | 41.6 ± 7.4 (13) | 43.9 ± 2.6 (20) | 41.5 ± 5.4 (15) | 41.8 ± 6.7 (13) | 45.2 ± 1.8 (16) | 40.6 ± 4.7 (15) | 43.6 ± 1.3 8 | 44.1 ± 2.4 5 |

Table 41: Hematology values measured in adult F1a rats following necropsy at PND90 (± 95% Confidence Interval). n=(x).

| Measurement | Parental treatment group | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| MCV $mm^3$ | 61.8 ± 1.4 (17) | 59.8 ± 1.6 (16) | **59.2 ± 1.3 (18)[1]** | **58.6 ± 1.8 (17)[1]** | 60.7 ± 1.5 (25) | 59.7 ± 1.5 (19) | 61.3 ± 2.5 (16) | 59.4 ± 2.0 (20) | **59.4 ± 1.8 (22)[1]** | **58.9 ± 1.8 (8)[2]** | 59 ± 2.3 6 |
| MCH pg | 20.4 ± .3 (15) | 18.1 ± 4.2 (8) | 19.8 ± .4 (14) | 20.6 ± 1 (13) | 20.4 ± .3 (20) | 20.7 ± .5 (15) | 20.2 ± .4 (13) | 19.8 ± .4 (16) | 20.4 ± .8 (15) | 19.7 ± .5 8 | 19.5 ± .4 5 |
| MCHC g/dl | 32.9 ± .8 (15) | 30.1 ± 7.1 (8) | 33.6 ± .9 (14) | 35.1 ± 2.5 (13) | 33.7 ± .9 (20) | 34.4 ± .6 (15) | 32.9 ± 1.6 (13) | 33.3 ± 1.2 (16) | 33.9 ± 1.7 (15) | 33.4 ± 1.6 8 | 33.1 ± 2.2 5 |
| RDW% | 12.1 ± .5 (17) | 12.2 ± .7 (16) | 12.5 ± .7 (18) | 12.4 ± .7 (17) | 12.5 ± .7 (25) | 12. ± .5 (19) | 13.4 ± 1.2 (16) | 12.7 ± 1 (20) | 13.1 ± .8 (22) | 12.3 ± .9 8 | 11.4 ± 1.2 6 |
| PLT $10^3$ µl | 978.4 ±148.3 (17) | 1064.6±1 19 (16) | 961.7 ± 95.5 (18) | 1013.6 ± 150 (17) | 988.8 ± 75.3 (25) | 918.1 ±137.8 (19) | 902.8 ±159.2 (16) | 939.7 ± 129 (20) | 884.7 ±152.2 (22) | 1044.1±1 05 8 | 1161.8 ± 92 6 |
| MPV fl | 8.6 ± .6 (17) | 8.3 ± .6 (16) | 8.8 ± .6 (18) | 8.6 ± .8 (17) | 8 ± .5 (25) | 8.4 ± .7 (19) | 8.0 ± .5 (16) | 8.12 ± .6 (20) | 8.35 ±.6 (22) | 7. 6 ± .7 8 | 7.5 ± .8 6 |

[1] Significantly different from sham surgery controls ($p \leq 0.05$)
[2] Significantly different from Group 11 (20 Ta steel pellets)

Table 42: Sperm motion parameters for F1a pups measured following euthanasia and necropsy on PND90

| Parental treatment group | F1a pups evaluated | % motile | % progressive | VAP[a] (μm/sec) | VSL[b] (μm/sec) | VCL[c] (μm/sec) | ALH[d] (μm) |
|---|---|---|---|---|---|---|---|
| Sham surgery | 6 | 46.5 ± 11.1 | 9.5 ± 5.1 | 147.8 ± 7.0 | 90.8 ± 11.3 | 361.8 ± 29.7 | 20.4 ± 1.6 |
| Ta steel pellets implanted in both parents | 13 | 47.9 ± 7.8 | 11.6 ± 4.1 | 155.1 ± 5.8 | 98.1 ± 5.4 | 372.0 ± 11.1 | 20.4 ± 1.2 |
| DU pellets implanted in male parents; Ta steel pellets implanted in female parents | 11 | 41.9 ± 6.5 | 7.8 ± 2.4 | 153.9 ± 6.2 | 92.6 ± 4.2 | 362.6 ± 10.8 | 20.3 ± 0.9 |
| Ta steel pellets implanted in male parents; DU pellets implanted in female parents | 35 | 44.7 ± 5.3 | 8.7 ± 1.7 | 151.5 ± 4.4 | 91.2 ± 3.5 | 364.1 ± 10.2 | 19.7 ± 0.7 |
| DU pellets implanted in both parents | 36 | 46.9 ± 3.8 | 10.2 ± 2.0 | 149.3 ± 2.8 | 92.0 ± 2.6 | 359.5 ± 8.1 | 19.7 ± 0.6 |

[a]VAP= Average path velocity; [b]VSL= Progressive straight line velocity; [c]VCL= Tract speed velocity; [d]ALH= Lateral sperm head displacement

Table 43: Average caudal sperm concentrations for F1a pups measured following euthanasia and necropsy on PND90

| Parental treatment group | F1a pups evaluated | # of Cells | Concentration ($10^6$ cells/mL) | Concentration ($10^6$ cells/gram tissue) |
|---|---|---|---|---|
| 1 | 14 | 35 ± 14.4 | 4.2 ± 1.6 | 284.0 ± 115.0 |
| 2 | 13 | 45.4 ± 12.8 | 5.1 ± 1.6 | 263.7 ± 92.6 |
| 3 | 10 | 63.6 ± 24.6 | 7.4 ± 2.9 | 413.8 ± 140.8 |
| 4 | 9 | 39.0 ± 16.1 | 4.7 ± 1.9 | 290.6 ± 124.8 |
| 5 | 13 | 33.0 ± 10.0 | 9.4 ± 12.5 | 466.6 ± 501.5 |
| 6 | 13 | 39.5 ± 13.9 | 4.8 ± 1.5 | 272.4 ± 79.0 |
| 7 | 13 | 39.8 ± 12.9 | 4.6 ± 1.5 | 296.8 ± 120.4 |
| 8 | 14 | 48.9 ± 26.3 | 10.6 ± 13.3 | 211.8 ± 53.5 |
| 9 | 12 | 45.2 ± 32.3 | 5.7 ± 3.7 | 255.5 ± 127.5 |
| 10 | 8 | 27.4 ± 8.7 | 3.2 ± 1.0 | 222.0 ± 61.5 |
| 11 | 3 | 24.0 ± 17.4 | 2.8 ± 2.0 | 226.0 ± 102.9 |
| 12 | 5 | 17.4 ± 13.3 | 2.0 ± 1.5 | 192.9 ± 115.8 |
| 13 | 8 | 28.4 ± 13.9 | 3.3 ± 1.6 | 220.2 ± 103.4 |

Table 44: Male F1a pup average PND20 and PND50 body weights and average PND20 – 50 weight gain

| Treatment Group (from Table 2) | N | PND20 Weight | PND50 Weight | Ave. Weight Gain PND20-50 |
|---|---|---|---|---|
| 1 | 3 | 49.4 ± 5.9 | 336.4 ± 119.1 | 287.0 ± 119.6 |
| 2 | 3 | 50.3 ± 12.8 | 340.9 ± 59.9 | 290.6 ± 72.6 |
| 3 | 4 | 51.1 ± 10.4 | 324.5 ± 80.9 | 273.4 ± 77.4 |
| 4 | 2 | 52.4 ± 13.5 | 342.5 ± 495.2 | 290.1 ± 481.7 |
| 5 | 4 | 49.9 ± 6.1 | 315.1 ± 92.6 | 265.2 ± 94.8 |
| 6 | 4 | 50.8 ± 6.9 | 348.2 ± 45.0 | 297.3 ± 47.7 |
| 7 | 5 | 48.7 ± 9.8 | 311.9 ± 65.7 | 263.2 ± 64.5 |
| 8 | 4 | 50.1 ± 5.7 | 275.4 ± 105.8 | 225.3 ± 101.6 |
| 9 | 4 | 50.3 ± 6.7 | 315.0 ± 66.8 | 264.7 ± 71.0 |

Table 45: Female F1a pup average PND20 and PND50 body weights and average PND20 – 50 weight gain

| Treatment Group (from Table 2) | N | PND20 Weight | PND50 Weight | Ave. Weight Gain PND20-50 |
|---|---|---|---|---|
| 1 | 4 | 47.2 ± 3.5 | 214.1 ± 33.3 | 166.9 ± 30.3 |
| 2 | 4 | 51.7 ± 3.9 | 214.4 ± 18.8 | 162.7 ± 17.7 |
| 3 | 4 | 49.5 ± 12.0 | 222.1 ± 39.9 | 172.5 ± 31.6 |
| 4 | 3 | 54.1 ± 5.6 | 211.4 ± 59.8 | 157.3 ± 60.9 |
| 5 | 4 | 48.2 ± 5.6 | 235.0 ± 64.7 | 186.8 ± 64.3 |
| 6 | 4 | 48.7 ± 6.2 | 198.9 ± 22.7 | 150.2 ± 20.0 |
| 7 | 5 | 46.1 ± 8.7 | 222.2 ± 19.9 | 176.1 ± 20.0 |
| 8 | 5 | 48.3 ± 6.5 | 218.0 ± 39.6 | 169.7 ± 37.7 |
| 9 | 4 | 47.6 ± 8.6 | 223.7 ± 22.3 | 176.2 ± 16.2 |

Table 46: Male reproductive success at 120-145 days post-implantation

| Treatment group | Mating success | Failure | Percent Mating Success | Average insemination time (days)* | 95% CI |
|---|---|---|---|---|---|
| Sham surgery | 19 | 2 | 91 | 2.2 | 0.6 |
| 12 Ta steel pellets | 63 | 7 | 90 | 3.0 | 0.8 |
| 12 DU pellets | 64 | 7 | 90 | 2.2 | 0.3 |
| 20 Ta steel pellets | 21 | 1 | 96 | 3.6 | 1.5 |
| 20 DU pellets | 21 | 3 | 87 | 2.5 | 0.8 |

*Number of days between introduction to female and appearance of vaginal plug in cage

Table 47: Gestation length and gestation weight gain of P1 females mated 120-145 days post-implantation surgery

| Treatment group | N | Gestation length (days) | N | Gestation weight gain (grams) |
|---|---|---|---|---|
| Sham surgery controls | 15 | 22.2 ± 0.2 | 15 | 132.8 ± 41.7 |
| 12 Ta steel pellets | 28 | 21.8 ± 0.3 | 28 | 125.6 ± 28 |
| 4 DU pellets | 28 | 22.2 ± 0.3 | 28 | 119.5 ± 26.9 |
| 8 DU pellets | 32 | **21.9 ± 0.3**[1] | 28 | 116.8 ± 14.3 |
| 12 DU pellets | 35 | 22.3 ± 0.5 | 33 | 147 ± 35.6 |
| 20 Ta steel pellets | 13 | 21.8 ± 0.9 | 13 | 121.8 ± 20.9 |
| 20 DU pellets | 23 | **21.7 ± 0.2**[1] | 23 | 116.4 ± 9.7 |

[1]Significantly different ($p \leq 0.05$) from sham surgery controls

Table 48: F1b generation litter size, percentage of males per litter, and pup survival for litters born to mothers mated at 120-145 days post-implantation day surgery (± 95% Confidence Interval)

| Parental Treatment Group | Number of litters | Litter size (PND1) | 95% CI | Percentage males | 95% CI | Percentage of pups surviving PND1-4 | 95% CI | Percentage of pups surviving PND5-20 | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 15 | 14.7 | 1.8 | 49.2 | 5.6 | 86.6 | 15.6 | 96.1 | 5.8 |
| 2 | 11 | 15.2 | 1.5 | 47.9 | 10.8 | 80.9 | 23.2 | 93.3 | 10.0 |
| 3 | 15 | 14.2 | 1.5 | 52.0 | 8.4 | 86.7 | 19.5 | 93.0 | 13.3 |
| 4 | 10 | 14.3 | 3.7 | 50.9 | 5.5 | 89.5 | 22.5 | 83.7 | 21.0 |
| 5 | 14 | 15.5 | 1.1 | 52.7 | 7.2 | 91.7 | 14.0 | 96.4 | 6.0 |
| 6 | 15 | 15.3 | 1.7 | 48.5 | 6.6 | 96.2 | 3.6 | 89.0 | 10.6 |
| 7 | 15 | 14.3 | 1.6 | 50.1 | 8.4 | 91.5 | 13.4 | 89.8 | 14.4 |
| 8 | 16 | 13.4 | 1.9 | 53.4 | 10.8 | 97.9 | 1.7 | 99.3 | 1.4 |
| 9 | 17 | 14.1 | 1.9 | 49.9 | 7.6 | 91.6 | 13.3 | 90.4 | 10.8 |
| 10 | 13 | 14.3 | 1.5 | 43.9 | 7.6 | 93.0 | 11.9 | 99.0 | 2.3 |
| 11 | 10 | 13.0 | 3.7 | 44.1 | 13.8 | 92.4 | 6.8 | 96.1 | 4.5 |
| 12 | 6 | 16.0 | 1.5 | **42.6**[1] | 8.7 | 97.1 | 3.4 | 100.0 | 0.0 |
| 13 | 9 | 14.1 | 1.6 | 59.1 | 13.6 | 84.9 | 28.8 | 100.0 | 0.0 |

[1]Significantly different than Group 11 (parents implanted with 20 Ta steel pellets only)

Table 49: F1b generation average litter size and pup weight at PND 1 and PND 4

| Parental Treatment Group | Litters evaluated | Average Litter Size PND1 | 95% CI | Average Pup Weight PND 1 (grams) | 95% CI | Average Litter Size PND4 | 95% CI | Average Pup Weight PND 4 (grams) | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 15 | 14.7 | 1.8 | 7.2 | 0.8 | 12.5 | 2.7 | 9.8 | 1.2 |
| 2 | 11 | 15.2 | 1.5 | 6.9 | 0.4 | 12.2 | 3.8 | 12.7 | 5.2 |
| 3 | 15 | 14.2 | 1.5 | 6.7 | 1.0 | 11.9 | 3.0 | 9.9 | 1.6 |
| 4 | 10 | 14.3 | 3.7 | 7.3 | 0.8 | 13.3 | 4.4 | 10.2 | 2.0 |
| 5 | 14 | 15.5 | 1.1 | 6.8 | 0.4 | 14.4 | 2.4 | 14.2 | 10.8 |
| 6 | 15 | 15.3 | 1.7 | 6.9 | 0.4 | 14.7 | 1.6 | 9.3 | 0.8 |
| 7 | 15 | 14.3 | 1.6 | 7.0 | 0.4 | 12.9 | 2.4 | 10.0 | 0.7 |
| 8 | 16 | 13.4 | 1.9 | 6.8 | 0.6 | 13.1 | 1.9 | 9.4 | 1.1 |
| 9 | 17 | 14.1 | 1.9 | 6.3 | 0.8 | 12.8 | 2.7 | 9.3 | 1.0 |
| 10 | 13 | 14.3 | 1.5 | 6.8 | 0.9 | 13.2 | 1.9 | 9.6 | 1.2 |
| 11 | 10 | 13.0 | 3.7 | 7.5 | 1.4 | 12.0 | 3.6 | 10.0 | 1.7 |
| 12 | 6 | 16.0 | 1.5 | 6.9 | 0.6 | 15.5 | 1.1 | **8.8**[1] | 2.4 |
| 13 | 9 | 14.1 | 1.6 | 6.7 | 1.0 | 12.1 | 4.3 | 9.9 | 1.6 |

[1]Significantly different than Group 11 (parents implanted with 20 Ta steel pellets only)

| Table 50: F1b average pup weight gain from PND5 though PND20 | | | | | |
|---|---|---|---|---|---|
| Parental treatment group (from Table 2) | Number of pups used in weight gain calculation (PND5-20) | Male Pup Weight Gain PND 5-20 (grams) | 95% CI | Female Pup Weight Gain PND 5-20 (grams) | 95% CI |
| 1 | 14 | 41.6 | 3.1 | 38.9 | 3.4 |
| 2 | 7 | 39.8 | 5.6 | 38.6 | 5.5 |
| 3 | 13 | 42.0 | 2.7 | 41.8 | 2.5 |
| 4 | 6 | 44.8 | 3.9 | 43.4 | 4.1 |
| 5 | 11 | 39.3 | 4.5 | 36.9 | 4.1 |
| 6 | 15 | 38.3 | 4.4 | 37.3 | 4.9 |
| 7 | 13 | 40.3 | 4.0 | 38.3 | 3.6 |
| 8 | 12 | 37.4 | 3.0 | 36.4 | 2.4 |
| 9 | 14 | 37.3 | 4.5 | 36.2 | 4.6 |
| 10 | 11 | 39.7 | 4.5 | 38.4 | 3.9 |
| 11 | 9 | 38.8 | 2.1 | 38.6 | 2.4 |
| 12 | 6 | 37.7 | 4.3 | 36.3 | 3.8 |
| 13 | 8 | 40.5 | 2.2 | 38.6 | 2.7 |

Table 51: P1 mother average time to find pup and retrieve to nest (maternal retrieval) on F1b PND2 for pups born to mothers mated at 30-45 days post-implantation day surgery (± 95% Confidence Interval)

| Treatment group | Number of animals tested | Mean (sec) | 95% CI |
|---|---|---|---|
| 1 | 8 | 94.8 | 33.7 |
| 2 | 8 | 89.7 | 15.0 |
| 3 | 8 | 83.3 | 16.7 |
| 4 | 8 | 128.6 | 23.8 |
| 5 | 8 | 141.8 | 34.4 |
| 6 | 8 | 168.3 | 28.9 |
| 7 | 8 | 92.8 | 15.7 |
| 8 | 8 | 85.3 | 30.5 |
| 9 | 8 | 102.4 | 24.4 |
| 10 | 10 | **87.0**[1] | **22.5** |
| 11 | 5 | 98.4 | 43.5 |
| 12 | 5 | 95.7 | 19.6 |
| 13 | 6 | **60.0**[1] | **19.9** |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

| Table 52: F1b average time to righting when assessed on PND4 (± 95% Confidence Interval) | | | |
|---|---|---|---|
| Parental treatment group | Number of animals tested | Mean (sec) | 95% CI |
| 1 | 20 | 8.4 | 2.1 |
| 2 | 20 | 8.7 | 1.9 |
| 3 | 20 | 13.4 | 13.5 |
| 4 | 20 | 7.5 | 2.4 |
| 5 | 20 | 9.5 | 2.3 |
| 6 | 20 | 9.5 | 2.6 |
| 7 | 20 | 7.3 | 2.5 |
| 8 | 20 | 9.3 | 3.3 |
| 9 | 20 | 8.2 | 2.3 |
| 10 | 21 | 12.5 | 2.4 |
| 11 | 11 | 10.0 | 2.9 |
| 12 | 15 | 12.9 | 5.8 |
| 13 | 9 | 12.7 | 4.1 |

Table 53: F1b vocalization frequency in response to separation from its P1 mother on F1b PND7 (± 95% Confidence Interval)

| Treatment group | Number of animals tested | Mean (vocs/min) | 95% CI |
|---|---|---|---|
| 1 | 14 | 33.5 | 12.0 |
| 2 | 14 | 26.1 | 10.3 |
| 3 | 14 | 29.3 | 10.4 |
| 4 | 14 | 24.6 | 16.3 |
| 5 | 14 | 44.4 | 14.8 |
| 6 | 14 | 48.1 | 25.0 |
| 7 | 14 | 53.1 | 18.8 |
| 8 | 14 | 30.0 | 11.2 |
| 9 | 14 | 27.7 | 14.0 |
| 10 | 10 | 40.9 | 15.2 |
| 11 | 14 | 22.6 | 7.6 |
| 12 | 17 | 40.1 | 12.9 |
| 13 | 22 | 20.6 | 7.2 |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

| Table 54: F1b PND17-32 juvenile play test results: dorsal contacts (± 95% Confidence Interval) | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of contacts) | 95% CI |
| 1 | 108 | 20.6 | 2.1 |
| 2 | 108 | 22.4 | 2.5 |
| 4 | 108 | 23.2 | 2.5 |
| 6 | 108 | 28.8 | 2.8 |
| 10 | 60 | 26.7 | 4.6 |
| 11 | 60 | 24.7 | 3.5 |
| 12 | 60 | 25.2 | 3.2 |
| 13 | 60 | 21.8 | 2.8 |

Table 55: F1b PND17-32 juvenile play test results: pins (± 95% Confidence Interval)

| Treatment group | Number of animals tested | Mean (# of pins) | 95% CI |
|---|---|---|---|
| 1 | 108 | 4.0 | 0.8 |
| 2 | 108 | 5.0 | 1.2 |
| 4 | 108 | 6.2 | 1.4 |
| 6 | 108 | 8.9 | 1.5 |
| 10 | 60 | 6.3 | 1.6 |
| 11 | 60 | 7.5 | 1.7 |
| 12 | 60 | 6.5 | 1.5 |
| 13 | 60 | 7.0 | 1.6 |

| Table 56: F1b PND17-32 juvenile play test results: pin latency in seconds (± 95% Confidence Interval) | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean pin latency (seconds) | 95% CI |
| 1 | 108 | 8.6 | 2.0 |
| 2 | 108 | 10.7 | 2.7 |
| 4 | 108 | 11.5 | 2.7 |
| 6 | 108 | 16.5 | 3.0 |
| 10 | 60 | 11.3 | 3.2 |
| 11 | 60 | 12.8 | 3.6 |
| 12 | 60 | 12.1 | 3.5 |
| 13 | 60 | 12.8 | 3.1 |

| Table 57: F1b Spontaneous Locomotor Activity assessment at PND32: distance traveled | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean distance traveled (cm) | 95% CI |
| 1 | 32 | 1065.4 | 104.4 |
| 2 | 32 | 1019.1 | 82.9 |
| 4 | 32 | 1113.7 | 93.2 |
| 6 | 32 | 1083.5 | 83.5 |
| 10 | 24 | 982.4 | 119.6 |
| 11 | 24 | 1043.7 | 148.5 |
| 12 | 24 | 1112.2 | 91.7 |
| 13 | 24 | 1056.4 | 86.9 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

Table 58: F1b Spontaneous Locomotor Activity assessment at PND32: time resting

| Treatment group | Number of animals tested | Mean time resting (seconds) | 95% CI |
|---|---|---|---|
| 1 | 32 | 19.7 | 1.6 |
| 2 | 32 | 22.0 | 3.3 |
| 4 | 32 | 20.3 | 1.4 |
| 6 | 32 | 24.9 | 5.7 |
| 10 | 24 | 108.2 | 17.6 |
| 11 | 24 | 112.4 | 19.2 |
| 12 | 24 | 98.1 | 6.0 |
| 13 | 24 | 101.7 | 7.8 |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

| Table 59: F1b Spontaneous Locomotor Activity assessment at PND32: stereotypical behavior count | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 32 | 173.5 | 5.6 |
| 2 | 32 | 167.3 | 12.1 |
| 4 | 32 | 173.7 | 5.2 |
| 6 | 32 | 168.3 | 7.8 |
| 10 | 24 | 173.6 | 15.8 |
| 11 | 24 | 168.5 | 17.3 |
| 12 | 24 | 181.6 | 5.5 |
| 13 | 24 | 178.8 | 6.8 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 60: F1b Spontaneous Locomotor Activity assessment at PND32: time ambulatory | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean time ambulatory (seconds) | 95% CI |
| 1 | 32 | 106.8 | 6.6 |
| 2 | 32 | 110.7 | 10.4 |
| 4 | 32 | 105.4 | 5.9 |
| 6 | 32 | 106.8 | 5.0 |
| 10 | 24 | 18.3 | 2.1 |
| 11 | 24 | 18.9 | 2.4 |
| 12 | 24 | 20.2 | 1.4 |
| 13 | 24 | 19.5 | 1.5 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 61: F1b Spontaneous Locomotor Activity assessment at PND32: stereotypical bursts | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 32 | 301.1 | 13.8 |
| 2 | 32 | 301.3 | 14.6 |
| 4 | 32 | 306.6 | 10.8 |
| 6 | 32 | 317.1 | 19.5 |
| 10 | 24 | 278.9 | 26.6 |
| 11 | 24 | 276.8 | 29.7 |
| 12 | 24 | 296.7 | 11.3 |
| 13 | 24 | 293.1 | 12.7 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 62: F1b Spontaneous Locomotor Activity assessment at PND32: horizontal movements | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 32 | 1052.3 | 93.0 |
| 2 | 32 | 1127.3 | 115.3 |
| 4 | 32 | 1217.8 | 76.1 |
| 6 | 32 | 1124.8 | 93.8 |
| 10 | 24 | 1465.5 | 124.5 |
| 11 | 24 | 1464.3 | 129.8 |
| 12 | 24 | 1611.8 | 122.2 |
| 13 | 24 | 1474.9 | 165.8 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 63: F1b Spontaneous Locomotor Activity assessment at PND32: ambulatory movements | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 32 | 761.0 | 74.0 |
| 2 | 32 | 830.9 | 100.6 |
| 4 | 32 | 912.4 | 63.4 |
| 6 | 32 | 836.6 | 74.4 |
| 10 | 24 | 1091.7 | 121.6 |
| 11 | 24 | 1147.9 | 114.4 |
| 12 | 24 | 1266.8 | 103.9 |
| 13 | 24 | 1139.0 | 148.0 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

Table 64: F1b Spontaneous Locomotor Activity assessment at PND32: # of rears

| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
|---|---|---|---|
| 1 | 32 | 14.9 | 6.7 |
| 2 | 32 | 18.6 | 7.2 |
| 4 | 32 | 25.3 | 11.4 |
| 6 | 32 | 18.0 | 7.3 |
| 10 | 24 | 26.0 | 12.2 |
| 11 | 24 | 56.6 | 20.3 |
| 12 | 24 | 51.5 | 19.4 |
| 13 | 24 | 52.0 | 16.6 |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

| Table 65: F1b Spontaneous Locomotor Activity assessment at PND32: # vertical plane movements | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Mean (# of behaviors) | 95% CI |
| 1 | 32 | 6.6 | 2.8 |
| 2 | 32 | 8.0 | 2.9 |
| 4 | 32 | 10.8 | 3.6 |
| 6 | 32 | 8.0 | 3.0 |
| 10 | 24 | 9.2 | 3.7 |
| 11 | 24 | 17.2 | 5.5 |
| 12 | 24 | 16.1 | 5.9 |
| 13 | 24 | 16.0 | 4.8 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

Table 66: F1b acoustic startle response magnitude when assessed at PND45-47

| Treatment group | Number of animals tested | Response magnitude (SDI units)[1] | 95% CI |
|---|---|---|---|
| 1 | 324 | 123.4 | 7.5 |
| 2 | 324 | 123.0 | 8.1 |
| 4 | 324 | 122.5 | 8.5 |
| 6 | 324 | 121.3 | 8.9 |
| 10 | 180 | 127.7 | 10.6 |
| 11 | 180 | 126.0 | 10.7 |
| 12 | 180 | 125.4 | 11.1 |
| 13 | 180 | 127.6 | 12.2 |

[1]San Diego Instrument startle response system units representing 1.22 millivolts – 5 volts divided by 4,095

| Table 67: F1b acoustic startle response latency when assessed at PND45-47 | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Response latency (seconds) | 95% CI |
| 1 | 324 | 68.2 | 8.9 |
| 2 | 324 | 82.0 | 10.3 |
| 4 | 324 | 72.5 | 9.6 |
| 6 | 324 | 87.6 | 11.5 |
| 10 | 180 | 72.0 | 9.8 |
| 11 | 180 | 60.0 | 7.2 |
| 12 | 180 | 74.4 | 9.6 |
| 13 | 180 | 76.8 | 10.4 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

| Table 68: F1b time to find the platform in the Morris Watermaze test at PND59-63 | | | |
|---|---|---|---|
| Treatment group | Number of animals tested | Time to find platform (seconds) | 95% CI |
| 1 | 36 | 45.8 | 17.3 |
| 2 | 36 | 27.5 | 8.8 |
| 4 | 36 | 24.7 | 10.1 |
| 6 | 36 | 42.4 | 17.2 |
| 10 | 36 | **18.5**[1] | **6.9** |
| 11 | 36 | 21.2 | 6.9 |
| 12 | 36 | 31.8 | 15.1 |
| 13 | 36 | 23.7 | 7.1 |
| [1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

Table 69: F1b mean path distance to find the platform in the Morris Watermaze test at PND59-63

| Treatment group | Number of animals tested | Path distance to platform (cm) | 95% CI |
|---|---|---|---|
| 1 | 36 | 739.3 | 348.7 |
| 2 | 36 | 471.2 | 150.6 |
| 4 | 36 | 370.5 | 149.4 |
| 6 | 36 | 604.5 | 224.6 |
| 10 | 36 | 293.7 | 107.7 |
| 11 | 36 | 352.9 | 99.0 |
| 12 | 36 | 454.1 | 192.8 |
| 13 | 36 | 369.0 | 112.8 |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

Table 70: F1b male pup weights at PND 60, 90, and 120

| Treatment group | N | PND20 | N | PND60 | N | PND90 | N | PND120 |
|---|---|---|---|---|---|---|---|---|
| 1 | 14 | 54.4 ± 4.0 | 9 | 355.4 ± 33.1 | 13 | 554.9 ± 31.0 | 6 | 680.5 ± 78.5 |
| 2 | 7 | 51.9 ± 7.5 | 7 | 373.4 ± 76.5 | 11 | 553.8 ± 46.6 | 9 | 610.0 ± 47.0 |
| 3 | 13 | 54.5 ± 3.4 | 8 | 395.5 ± 27.1 | 12 | 535.7 ± 37.5 | 8 | 609.7 ± 27.1 |
| 4 | 6 | 59.2 ± 4.2 | 6 | 381.8 ± 53.6 | 9 | 529.0 ± 69.4 | 7 | 612.5 ± 56.6 |
| 5 | 11 | 50.9 ± 4.6 | 11 | 391.5 ± 40.4 | 12 | 552.0 ± 47.8 | 9 | 661.1 ± 58.2 |
| 6 | 15 | 50.1 ± 5.4 | 5 | 387.1 ± 17.6 | 11 | 521.9 ± 39.8 | 10 | 647.8 ± 63.3 |
| 7 | 13 | 52.2 ± 5.1 | 9 | 409.2 ± 41.1 | 11 | 526.4 ± 45.4 | 7 | 602.1 ± 92.7 |
| 8 | 12 | 49.2 ± 4.2 | 8 | 404.9 ± 50.0 | 14 | 531.7 ± 42.8 | 10 | 646.2 ± 59.1 |
| 9 | 14 | 48.4 ± 4.9 | 10 | 390.2 ± 45.6 | 12 | 525.3 ± 43.4 | 9 | 626.0 ± 73.1 |
| 10 | 11 | 51.8 ± 5.1 | 15 | 269.3 ± 43.1 | 1 | 527.3 | 2 | 559.6 ± 125.8 |
| 11 | 9 | 50.3 ± 3.2 | 7 | 290.6 ± 80.9 | 1 | 509.4 | 5 | 611.1 ± 74.4 |
| 12 | 6 | 49.7 ± 4.1 | 5 | 240.8 ± 68.6 | 1 | 559.6 | - | - |
| 13 | 8 | 52.6 ± 3.1 | 4 | 291.4 ± 97.3 | 2 | 479.0 ± 550.2 | 1 | 680.4 |

| Table 71: F1b male pup weight gain PND 20-60, 20-90, 20-120 | | | |
|---|---|---|---|
| Treatment group | PND 20-60 | PND 20-90 | PND 20-120 |
| 1 | 302.6 ± 35.9 | 500.6 ± 28.9 | 626.1 ± 74.0 |
| 2 | 319.2 ± 71.6 | 500.2 ± 47.6 | 555.7 ± 47.8 |
| 3 | 337.7 ± 28.7 | 480.9 ± 36.8 | 556.6 ± 26.1 |
| 4 | 326.0 ± 50.5 | 471.4 ± 66.1 | 556.8 ± 55.7 |
| 5 | 335.5 ± 37.6 | 500.6 ± 45.9 | 609.1 ± 56.4 |
| 6 | 331.7 ± 12.5 | 472.4 ± 38.1 | 595.2 ± 60.2 |
| 7 | 359.5 ± 38.5 | 473.2 ± 44.8 | 552.2 ± 91.4 |
| 8 | 352.4 ± 48.1 | 481.4 ± 41.5 | 594.4 ± 75.7 |
| 9 | 336.5 ± 40.7 | 474.6 ± 39.1 | 582.7 ± 77.6 |
| 10 | 240.2 ± 40.4 | 475.5 | 507.5 ± 122.0 |
| 11 | 240.4 ± 82.4 | 459.2 | 560.0 ± 68.6 |
| 12 | 188.4 ± 66.7 | 511.7 | - |
| 13 | 237.3 ± 92.7 | 433.2 ± 550.2 | 625.2 |

Table 72: F1b female pup weights at PND 60, 90, and 120

| Treatment group | N | PND20 | N | PND60 | N | PND90 | N | PND120 |
|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 50.5 ± 4.2 | 9 | 203.2 ± 36.5 | 17 | 288.3 ± 15.9 | 8 | 360.1 ± 46.2 |
| 2 | 7 | 50.2 ± 7.3 | 10 | 239.5 ± 19.1 | 12 | 285.1 ± 21.1 | 9 | 336.7 ± 20.3 |
| 3 | 13 | 54.4 ± 3.3 | 10 | 254.6 ± 24.6 | 16 | 300.0 ± 20.5 | 11 | 357.3 ± 26.8 |
| 4 | 6 | 57.2 ± 5.2 | 10 | **264.7 ± 19.6**[1] | 14 | 294.9 ± 19.4 | 7 | 371.2 ± 42.6 |
| 5 | 11 | 47.7 ± 4.0 | 10 | 244.1 ± 21.0 | 15 | 292.8 ± 18.6 | 8 | 341.2 ± 19.0 |
| 6 | 15 | 48.4 ± 5.5 | 11 | 238.9 ± 21.4 | 16 | 301.1 ± 19.3 | 10 | 365.0 ± 24.9 |
| 7 | 13 | 49.6 ± 4.6 | 5 | 231.6 ± 58.9 | 12 | 284.6 ± 20.6 | 10 | 318.2 ± 28.0 |
| 8 | 11 | 47.4 ± 3.0 | 12 | **254.8 ± 20.8**[1] | 17 | 286.7 ± 18.2 | 9 | 355.3 ± 29.2 |
| 9 | 14 | 46.9 ± 5.2 | 13 | **259.1 ± 15.5**[1] | 19 | 293.7 ± 16.0 | 12 | 347.9 ± 35.1 |
| 10 | 12 | 49.8 ± 4.4 | 16 | 209.6 ± 29.1 | 15 | **267.5 ± 13.6**[2] | - | - |
| 11 | 10 | 50.5 ± 2.3 | 10 | 207.1 ± 47.5 | 12 | 290.9 ± 11.4 | 6 | 344.4 ± 24.3 |
| 12 | 6 | 47.6 ± 4.2 | 5 | 190.0 ± 25.5 | 10 | 267.8 ± 16.3 | - | - |
| 13 | 8 | 50.5 ± 4.2 | 6 | 212.3 ± 18.7 | 10 | 269.3 ± 16.9 | 4 | 360.3 ± 92.5 |

[1]Significantly different ($p \leq 0.05$) from Group 1 sham surgery controls
[2]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

| Table 73: F1b female pup weight gain PND 20-60, 20-90, 20-120 | | | |
|---|---|---|---|
| Treatment Group | PND 20-60 | PND 20-90 | PND 20-120 |
| 1 | 160.9 ± 37.0 | 238.8 ± 13.8 | 308.6 ± 44.2 |
| 2 | 186.9 ± 19.3 | 224.2 ± 14.4 | 284.7 ± 19.0 |
| 3 | 194.3 ± 22.4 | 245.1 ± 19.4 | 305.0 ± 26.6 |
| 4 | 205.8 ± 14.4 | 246.4 ± 16.9 | 332.1 ± 33.2 |
| 5 | 191.4 ± 18.8 | 240.7 ± 14.9 | 291.1 ± 18.0 |
| 6 | 191.4 ± 20.2 | 250.1 ± 18.2 | 314.0 ± 23.3 |
| 7 | 176.7 ± 64.7 | 233.6 ± 16.5 | 267.7 ± 23.6 |
| 8 | 206.9 ± 20.0 | 236.2 ± 18.4 | 301.6 ± 27.5 |
| 9 | 206.8 ± 13.3 | 243.1 ± 13.8 | 304.2 ± 32.0 |
| 10 | 161.4 ± 28.5 | 220.1 ± 12.0 | - |
| 11 | 140.9 ± 35.3 | 241.1 ± 11.2 | 292.0 ± 25.1 |
| 12 | 140.0 ± 26.0 | 220.8 ± 15.5 | 296.8 |
| 13 | 160.9 ± 18.0 | 221.1 ± 15.6 | 311.7 ± 80.8 |
| [1]Significantly different ($p \leq 0.05$) from Group 1 sham surgery controls<br>[2]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls | | | |

Table 74: Hematology values measured in adult F1b rats following necropsy at 200 days post-implantation (± 95% Confidence Interval). n=(x).

| Endpoint | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WBC ($10^3$ µl) | 12.1 ± 2.6 (36) | 11.2±2.8 (24) | 10.2 ± 1.9 (32) | 11.4 ± 2.2 (40) | 13 ± 5.1 (23) | 10.2 ± 1.3 (31) | 10.7 ± 2.9 (23) | 9.6 ± .7 (33) | 9.3 ± 1.1 (31) | **12.3 ± 1.1 (38)[1]** | 10.5 ± .9 (29) |
| % Lymphocytes | 85 ± 2.0 (35) | 84.8 ± 2.8 (24) | 84.8 ± 2.2 (32) | 83.9 ± 2.7 (38) | 85.9 ± 2.4 (22) | 84.6 ± 2.7 (31) | 87.0 ± 2.3 (23) | 86.4 ± 1.5 (32) | 84.4 ± 2.4 (29) | 85.4 ± 2 (39) | 83.4 ± 3.8 (31) |
| % Monocytes | 7 ± .7 (35) | 7.3 ± .8 (24) | 6.9 ± .9 (32) | 7.8 ± 1 (38) | 7.4 ± 1.1 (22) | 6.7 ± .9 (31) | 6 ± .8 (23) | 6.7 ± .6 (32) | 7.3 ± .9 (29) | 7 ± .9 (39) | 7.2 ± .8 (30) |
| % Granulocytes | 8 ± 1.7 (35) | 7.9 ± 2.4 (24) | 8.3 ± 1.6 (32) | 7.5 ± 1.6 (38) | 6.5 ± 1.7 (23) | 8.7 ± 2.1 (31) | 7 ± 1.9 (23) | 6.9 ± 1.3 (32) | 8.2 ± 1.9 (29) | 7.7 ± 1.3 (39) | 7.7 ± 1.5 (30) |
| # Lymphocytes | 8.9 ± 1.2 (34) | 9.3 ± 1.7 (24) | 8.5 ± 1.3 (32) | 8.7 ± .7 (38) | 9.1 ± 1.6 (22) | 8.4 ± .8 (31) | 9.1 ± 2.1 (23) | 8.3 ± .7 (32) | 8.3 ± .7 (29) | 10.5 ± 1 (38) | 9 ± .8 (29) |
| # Monocytes | .8 ± .2 (34) | .8 ± .2 (24) | .7 ± .2 (32) | .8 ± .1 (38) | .8 ± .2 (22) | .7 ± .2 (31) | .7 ± .2 (22) | .6 ± .1 (32) | .73 ± .1 (29) | .8 ± .1 (38) | .7 ± .1 (29) |
| # Granulocytes | 1 ± .3 (34) | 1.1 ± 1 (24) | 1 ± .5 (32) | .8 ± .3 (38) | .9 ± .5 (21) | 1.0 ± .4 (31) | .9 ± .7 (23) | .6 ± .1 (32) | .8 ± .2 (29) | .9 ± .2 (38) | .8 ± .2 (29) |
| RBC ($10^6$ µl) | 7.1 ± .6 (22) | 7.5 ± .3 (10) | 7.3 ± .3 (15) | 7.5 ± .2 (21) | 7.3 ± 1 (14) | 7.6 ± .1 (18) | 7.7 ± .1 (14) | 7.5 ± .3 (15) | 6.5 ± 1.8 (12) | 7.6 ± .4 (10) | 7 ± 1.2 (8) |
| HgB (g/dl) | 15 ± .8 (43) | 15.6 ± .4 (27) | 15.5 ± .3 (33) | 15.4 ± .6 (44) | 15.1 ± 1.1 (25) | 15.4 ± .3 (34) | 15.4 ± .3 (23) | 15.2 ± .4 (33) | 14.9 ± 1.3 (31) | 16 ± .3 (39) | 16.0 ± .5 (34) |
| Hematocrit | 42.1 ± | 43.6 ± | 43.9 ± | 44.2 ± | 42.1 ± | 43.6 ± | 43.9 ± | 42 ± 1.5 | 38.6 ± | 44.9 ± | 41.5 ± |

Table 74: Hematology values measured in adult F1b rats following necropsy at 200 days post-implantation (± 95% Confidence Interval). n=(x).

| Endpoint | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| % | 3.7 (22) | 2.1 (10) | 1.6 (15) | 1.5 (21) | 5.9 (14) | 1.2 (18) | 1.5 (14) | (15) | 10.6 (12) | 3.1 (10) | 6.4 (8) |
| MCV $mm^3$ | 58 ± 1.2 (42) | 56.9 ± 1.4 (27) | 57.1 ± 1.3 (33) | 57.5 ± 1.2 (44) | 57.8 ± 1.6 (25) | 56.7 ± 1.0 (34) | 56.9 ± 1.7 (23) | 57 ± 2.2 (33) | 56.8 ± 1.4 (30) | 56.9 ± 1.2 (38) | 57.8 ± 2.5 (32) |
| MCH (pg) | 19.6 ± 1.2 (22) | 19.8 ± .7 (10) | 20.5 ± .7 (15) | 19.3 ± 1.3 (21) | 19.9 ± .6 (14) | 19.8 ± .5 (18) | 19.5 ± .4 (14) | 19.5 ± .5 (15) | 21.2 ± 1.9 (12) | 19.8 ± .6 (10) | 22.0 ± 5.6 (8) |
| MCHC (g/dl) | 32 ± 3.2 (22) | 33.9 ± .9 (10) | 34.6 ± .8 (15) | 32.8 ± 2.3 (21) | 34.6 ± 1.1 (14) | 34.5 ± .6 (18) | 34.4 ± 1.1 (14) | 34.5 ± .6 (15) | 36.6 ± 4.8 (12) | **33.7 ± .7 (10)[1]** | 36 ± 2.5 (8) |
| RDW% | 13.7 ± .6 (43) | 14.2 ± 1.3 (27) | 13.2 ± .4 (33) | 13.6 ± .5 (43) | 13.8 ± .7 (25) | 13.5 ± .4 (34) | 14.1 ± .8 (23) | 14 ± .7 (33) | 14.3 ± 1.5 (31) | 14.4 ± 1.2 (39) | 14.4 ± 1.1 (34) |
| PLT ($10^3$ µl) | 1044 ± 72 (43) | 1070.9 ± 62.6 (27) | 1060.9 ± 61.8 (33) | 1023.4 ± 103.3 (43) | 1062.3 ± 113.1 (24) | 1031.3 ± 53.4 (34) | 991 ± 64.9 (22) | 1073.4 ± 133.7 (31) | 960.5 ± 98.4 (31) | 1017.4 ± 57 (37) | 969.1 ± 89.7 (34) |
| MPV (fl) | 7.7 ± .2 (43) | 7.7 ± .3 (27) | 7.3 ± .2 (33) | 8 ± .3 (43) | 7.9 ± .3 (24) | 7.5 ± .23 (34) | 7.7 ± .4 (22) | 7.5 ± .2 (31) | 7.4 ± .3 (30) | 7.9 ± .4 (37) | 7.6 ± .2 (33) |

[1]Significantly ($p \leq 0.05$) different from Group 11 (inert implant controls)

Table 75: Sperm motion parameters for F1b pups measured following euthanasia and necropsy on PND200

| Parental treatment group | F1b pups evaluated | % motile | % progressive | VAP[a] (μm/sec) | VSL[b] (μm/sec) | VCL[c] (μm/sec) | ALH[d] (μm) |
|---|---|---|---|---|---|---|---|
| 1 | 24 | 71.6±7.3 | 22.5±3.3 | 170.0±17.0 | 118.1±12.1 | 322.0±32.4 | 14.5±1.4 |
| 2 | 17 | 76.9±5.5 | 23.6±4.1 | 185.2±14.0 | 127.3±11.9 | 354.9±26.2 | 15.8±1.4 |
| 3 | 17 | 72.4±7.5 | 20.9±3.8 | 166.8±21.6 | 112.6±16.0 | 332.9±38.3 | 14.8±1.8 |
| 4 | 19 | 73.9±3.6 | 25.0±3.1 | 185.2±13.0 | 129.4±9.4 | 356.5±16.2 | 15.7±0.6 |
| 5 | 15 | 73.3±12.4 | 24.3±4.9 | 169.6±31.9 | 119.6±22.2 | 326.4±55.5 | 14.8±2.4 |
| 6 | 22 | 73.4±5.3 | 23.5±3.0 | 176.5±11.6 | 122.7±8.3 | 343.1±24.6 | 15.4±1.3 |
| 7 | 18 | 75.5±4.9 | 23.5±4.0 | 170.1±11.1 | 118.6±8.5 | 348.1±17.0 | 15.8±0.9 |
| 8 | 25 | 76.0±4.2 | 23.6±2.7 | 167.3±13.8 | 115.8±9.6 | 334.1±23.5 | 14.8±1.1 |
| 9 | 19 | 76.5±4.6 | 21.3±3.2 | 173.5±16.8 | 117.5±12.4 | 333.7±29.3 | 15.1±1.3 |
| 10 | 27 | 77.4±3.1 | 26.2±2.1 | 181.7±10.0 | 127.3±6.5 | 346.3±16.3 | 15.3±0.8 |
| 11 | 20 | 78.5±3.8 | 25.7±2.0 | 180.9±11.4 | 126.9±7.8 | 337.6±17.0 | 14.9±0.7 |

[a]VAP= Average path velocity; [b]VSL= Progressive straight line velocity; [c]VCL= Tract speed velocity; [d]ALH= Lateral sperm head displacement

Table 76: Average caudal sperm concentrations for F1b pups measured following euthanasia and necropsy on PND200

| Parental treatment group | F1b pups evaluated | # of Cells | Concentration ($10^6$ cells/mL) | Concentration ($10^6$ cells/gram tissue) |
|---|---|---|---|---|
| 1 | 21 | 131.1 ± 35.5 | 15.2 ± 4.1 | 559.2 ± 139.4 |
| 2 | 16 | 138.3 ± 31.0 | 16.0 ± 3.6 | 703.4 ± 154.3 |
| 3 | 13 | 106.8 ± 32.1 | 12.4 ± 3.7 | 491.9 ± 164.5 |
| 4 | 11 | 119.4 ± 37.0 | 13.8 ± 4.3 | 586.5 ± 171.3 |
| 5 | 15 | 125.8 ± 3 8.1 | 14.6 ± 4.4 | 617.9 ± 153.9 |
| 6 | 13 | 132.4 ± 31.2 | 15.3 ± 3.6 | 652.8 ± 127.5 |
| 7 | 19 | 132.7 ± 46.9 | 15.4 ± 5.4 | 600.6 ± 163.1 |
| 8 | 14 | 122.3 ± 28.6 | 14.1 ± 3.3 | 651.2 ± 140.5 |
| 9 | 23 | 123.6 ± 48.0 | 14.3 ± 5.6 | 522.9 ± 186.7 |
| 10 | 23 | 169.5 ± 42.5 | 19.6 ± 4.9 | 778.2 ± 211.2 |
| 11 | 5 | 208.8 ± 50.2 | 24.1 ± 5.8 | 1037.1 ± 344.1 |
| 12 | 8 | 190.9 ± 47.8 | 22.1 ± 5.5 | 930.4 ± 263.5 |
| 13 | 17 | 242.5 ± 35.3 | 28.1 ± 4.1 | 1111.4 ± 199.8 |

Table 77: Mean absolute weights (± 95% confidence interval) in grams of major organs and organ systems of male F1b adult rats that underwent necropsy on PND200. n=(x).

| Parental treatment group | Sex organs | Liver | Kidneys | Heart | Spleen | Brain |
|---|---|---|---|---|---|---|
| 1 | 10.8 ± 1.0 (20) | 23.5 ± 1.7 (21) | 4.7 ± 0.4 (20) | 2.2 ± 0.2 (21) | 1.1 ± 0.1 (20) | 2.1 ± 0.1 (21) |
| 2 | 11.2 ± 0.8 (8) | 21.7 ± 3.2 (13) | 4.7 ± 0.5 (13) | 2.3 ± 0.4 (14) | 0.9 ± 0.2 (12) | 2.1 ± 0.1 (14) |
| 3 | 10.5 ± 1.3 (12) | 23.9 ± 2.2 (14) | 4.7 ± 0.3 (13) | 2.3 ± 0.2 (14) | 1.0 ± 0.1 (14) | 2.2 ± 0.1 (14) |
| 4 | 11.1 ± 1.1 (13) | 23.4 ± 2.1 (14) | 4.7 ± 0.4 (14) | 2.3 ± 0.3 (13) | 0.9 ± 0.2 (14) | 2.1 ± 0.2 (14) |
| 5 | 11.1 ± 1.1 (9) | 26.2 ± 2.7 (13) | 5.0 ± 0.4 (13) | 2.2 ± 0.3 (13) | 1.0 ± 0.1 (13) | 2.1 ± 0.1 (12) |
| 6 | 10.8 ± 1.0 (15) | 23.0 ± 2.0 (13) | 5.0 ± 0.3 (15) | 2.4 ± 0.3 (14) | 0.9 ± 0.1 (14) | 2.1 ± 0.1 (15) |
| 7 | 11.1 ± 1.0 (12) | 22.4 ± 2.0 (13) | 4.8 ± 0.4 (13) | 2.1 ± 0.3 (13) | 1.0 ± 0.1 (12) | 2.0 ± 0.1 (13) |
| 8 | 11.2 ± 1.1 (18) | 23.4 ± 1.9 (19) | 5.0 ± 0.3 (19) | 2.2 ± 0.2 (19) | 0.9 ± 0.1 (19) | 2.0 ± 0.1 (19) |
| 9 | 11.1 ± 1.0 (15) | 24.1 ± 2.2 (16) | 5.0 ± 0.4 (14) | 2.4 ± 0.2 (16) | 1.0 ± 0.1 (16) | 2.2 ± 0.1 (16) |
| 10 | 9.5 ± 0.8 (16) | 23.3 ± 1.2 (16) | 4.6 ± 0.3 (16) | 2.2 ± 0.1 (16) | 0.9 ± 0.1 (16) | 2.2 ± 0.1 (15) |
| 11 | 9.9 ± 0.6 (15) | 23.9 ± 2.2 (14) | 4.6 ± 0.4 (15) | 2.5 ± 0.2 (15) | 0.9 ± 0.2 (15) | 2.1 ± 0.1 (15) |

Table 78: Mean relative weights ($10^{-3}$ ± 95% confidence interval) in grams of major organs and organ systems of male F1b adult rats that underwent necropsy on PND200. n=(x).

| Parental treatment group | Sex organs | Liver | Kidneys | Heart | Spleen | Brain |
|---|---|---|---|---|---|---|
| 1 | 24.2 ± 8.9 (9) | 50.1 ± 18.1 (10) | 10.7 ± 4.2 (9) | 4.3 ± 1.1 (10) | 2.3 ± 0.8 (10) | 4.0 ± 0.9 (10) |
| 2 | 20.0 ± 2.9 (5) | 38.5 ± 7.2 (9) | 8.8 ± 1.3 (8) | 3.9 ± 0.5 (9) | 1.6 ± 0.4 (8) | 3.8 ± 0.5 (9) |
| 3 | 22.2 ± 5.7 (9) | 46.7 ± 11.6 (11) | 9.9 ± 2.8 (10) | 4.9 ± 1.3 (11) | 2.0 ± 0.4 (11) | 4.5 ± 0.9 (11) |
| 4 | 21.8 ± 4.4 (9) | 47.0 ± 11.3 (9) | 9.2 ± 2.8 (9) | 4.7 ± 1.8 (8) | 1.7 ± 0.4 (9) | 4.2 ± 1.2 (9) |
| 5 | 20.6 ± 3.0 (8) | 47.4 ± 3.1 (12) | 9.0 ± 0.9 (12) | 4.1 ± 0.5 (12) | 1.8 ± 0.2 (12) | 3.8 ± 0.4 (11) |
| 6 | 19.7 ± 2.2 (9) | 43.9 ± 4.3 (7) | 9.3 ± 1.1 (9) | 4.5 ± 0.5 (8) | 2.0 ± 0.3 (9) | 4.0 ± 0.6 (9) |
| 7 | 21.7 ± 3.7 (9) | 43.0 ± 3.4 (10) | 9.2 ± 1.5 (10) | 4.1 ± 1.0 (10) | 2.0 ± 0.4 (10) | 3.9 ± 0.4 (10) |
| 8 | 22.0 ± 3.1 (12) | 44.5 ± 2.9 (12) | 9.6 ± 0.9 (13) | 4.1 ± 0.4 (13) | 1.8 ± 0.3 (13) | 3.9 ± 0.4 (12) |
| 9 | 19.6 ± 1.6 (9) | 41.9 ± 2.6 (10) | 9.1 ± 0.7 (10) | 4.3 ± 0.8 (10) | 1.8 ± 0.3 (10) | 4.0 ± 0.6 (10) |
| 10 | 22.2 ± 10.8 (3) | 60.0 ± 38.9 (3) | 12.6 ± 14.0 (3) | 5.8 ± 4.9 (3) | 2.4 ± 1.8 (3) | 5.5 ± 3.9 (3) |
| 11 | 22.2 ± 7.4 (2) | 61.4 ± NA (1) | 11.5 ± 13.2 (2) | 6.0 ± 0.6 (2) | 2.7 ± 3.9 (2) | 4.5 ± 0.4 (2) |

Table 79: Mean absolute weights (± 95% confidence interval) in grams of major organs and organ systems of female F1b adult rats that underwent necropsy on PND200. n=(x).

| Parental treatment group | Sex organs | Liver | Kidneys | Heart | Spleen | Brain |
|---|---|---|---|---|---|---|
| 1 | 1.2 ± 0.2 (21) | 13.7 ± 1.0 (21) | 2.9 ± 0.2 (21) | 1.8 ± 0.2 (16) | 0.7 ± 0.1 (21) | 1.8 ± 0.1 (21) |
| 2 | 1.1 ± 0.2 (13) | 14.0 ± 1.1 (14) | 3.0 ± 0.4 (16) | 1.8 ± 0.2 (20) | 0.7 ± 0.1 (16) | 1.7 ± 0.1 (16) |
| 3 | 1.1 ± 0.1 (19) | 14.1 ± 1.3 (20) | 2.8 ± 0.1 (20) | 1.8 ± 0.2 (20) | 0.8 ± 0.1 (20) | 1.7 ± 0.1 (20) |
| 4 | 1.2 ± 0.2 (16) | 13.7 ± 1.0 (16) | 3.0 ± 0.2 (16) | 1.9 ± 0.2 (16) | 0.7 ± 0.1 (16) | 1.7 ± 0.2 (15) |
| 5 | 1.2 ± 0.1 (15) | 14.2 ± 1.5 (14) | 2.8 ± 0.2 (15) | 2.0 ± 0.3 (15) | 0.8 ± 0.2 (14) | 1.7 ± 0.1 (15) |
| 6 | 1.1 ± 0.2 (15) | 14.5 ± 1.4 (15) | 3.0 ± 0.3 (15) | 2.0 ± 0.3 (15) | 0.7 ± 0.1 (15) | 1.7 ± 0.1 (15) |
| 7 | 1.2 ± 0.2 (15) | 13.4 ± 1.1 (14) | 2.9 ± 0.2 (15) | 1.9 ± 0.2 (15) | 0.7 ± 0.1 (15) | 1.8 ± 0.1 (15) |
| 8 | 1.3 ± 0.2 (17) | 13.4 ± 1.3 (16) | 2.8 ± 0.3 (17) | 1.6 ± 0.2 (17) | 0.7 ± 0.2 (16) | 1.7 ± 0.1 (16) |
| 9 | 1.1 ± 0.2 (16) | 14.1 ± 1.2 (17) | 3.0 ± 0.2 (17) | 1.9 ± 0.2 (17) | 0.8 ± 0.1 (17) | 1.8 ± 0.1 (17) |
| 10 | 1.3 ± 0.2 (18) | 14.4 ± 1.0 (20) | 3.0 ± 0.2 (20) | 2.2 ± 0.2 (20) | 0.8 ± 0.1 (20) | 1.9 ± 0.1 (20) |
| 11 | 1.3 ± 0.2 (16) | 15.7 ± 1.1 (17) | 3.1 ± 0.3 (17) | 2.3 ± 0.2 (17) | 0.8 ± 0.2 (17) | 1.8 ± 0.2 (17) |

Table 80: Mean relative weights ($10^{-3}$ ± 95% confidence interval) in grams of major organs and organ systems of female F1b adult rats that underwent necropsy on PND200. n=(x).

| Parental treatment group | Sex organs | Liver | Kidneys | Heart | Spleen | Brain |
|---|---|---|---|---|---|---|
| 1 | 4.2 ± 0.8 (15) | 45.2 ± 3.2 (15) | 10.1 ± 0.8 (15) | 6.2 ± 0.7 (15) | 2.2 ± 0.4 (15) | 6.2 ± 0.9 (15) |
| 2 | 3.6 ± 0.7 (9) | 49.3 ± 5.3 (9) | 9.3 ± 0.7 (9) | 6.1 ± 0.8 (11) | 2.3 ± 0.4 (11) | 5.9 ± 0.7 (11) |
| 3 | 3.7 ± 0.6 (15) | 47.8 ± 4.3 (15) | 9.4 ± 0.4 (15) | 6.2 ± 0.5 (15) | 2.7 ± 0.4 (15) | 6.0 ± 0.5 (15) |
| 4 | 3.9 ± 0.8 (14) | 46.0 ± 3.7 (14) | 10.0 ± 1.0 (14) | 6.4 ± 0.9 (14) | 2.1 ± 0.3 (13) | 5.8 ± 0.7 (13) |
| 5 | 3.9 ± 0.6 (13) | 46.1 ± 4.2 (12) | 9.4 ± 0.6 (13) | 6.5 ± 1.2 (12) | 2.3 ± 0.5 (12) | 5.8 ± 0.5 (13) |
| 6 | 3.6 ± 0.7 (15) | 45.9 ± 3.3 (15) | 9.7 ± 0.6 (13) | 6.1 ± 0.6 (14) | 2.3 ± 0.4 (15) | 5.6 ± 0.7 (15) |
| 7 | 4.3 ± 0.7 (11) | 47.2 ± 5.1 (10) | 10.4 ± 0.6 (11) | 5.9 ± 0.6 (10) | 2.4 ± 0.5 (11) | 6.5 ± 0.7 (11) |
| 8 | 4.6 ± 0.8 (15) | 45.6 ± 3.3 (14) | 9.6 ± 0.7 (15) | 5.6 ± 0.6 (15) | 2.3 ± 0.5 (14) | 5.7 ± 0.5 (14) |
| 9 | 3.7 ± 0.6 (15) | 48.3 ± 3.5 (16) | 10.3 ± 0.6 (16) | 6.6 ± 0.7 (16) | 2.6 ± 0.4 (16) | 6.2 ± 0.7 (15) |
| 10 | 4.7 ± 0.9 (13) | 53.3 ± 2.8 (15) | 11.0 ± 0.7 (14) | 7.9 ± 0.7 (15) | 2.8 ± 0.4 (14) | 7.0 ± 0.4 (14) |
| 11 | 4.2 ± 0.8 (11) | 51.8 ± 5.5 (10) | 10.7 ± 1.2 (11) | 8.0 ± 1.0 (11) | 2.6 ± 0.8 (10) | 6.6 ± 0.8 (11) |

Table 81: F1b generation reproductive success when mated at PND70

| Parental treatment group | Mating pairs (N) | Mating success | Failure | Percent Mating Success | Average insemination time (days)* | 95% CI |
|---|---|---|---|---|---|---|
| 1 | 24 | 15 | 9 | 63 | 2.7 | 0.9 |
| 2 | 21 | 12 | 9 | 57 | 2.8 | 0.6 |
| 3 | 21 | 14 | 7 | 67 | 4.2 | 1.9 |
| 4 | 21 | 12 | 9 | 57 | 2.6 | 0.6 |
| 5 | 21 | 13 | 8 | 62 | 3.5 | 1.4 |
| 6 | 21 | 13 | 8 | 62 | 3.8 | 1.8 |
| 7 | 20 | 14 | 6 | 70 | 3.7 | 1.8 |
| 8 | 21 | 16 | 5 | 76 | 3.6 | 1.4 |
| 9 | 21 | 15 | 6 | 71 | 3.9 | 1.3 |
| 10 | 25 | 18 | 7 | 72 | 3.0 | 1.0 |
| 11 | 19 | 16 | 3 | 84 | 4.7 | 2.0 |
| 12 | 11 | 10 | 1 | 91 | 2.7 | 1.2 |
| 13 | 15 | 11 | 4 | 73 | 3.8 | 2.3 |

*Number of days between introduction to female and appearance of vaginal plug in cage

| Table 82: Gestation length and gestation weight gain of F1b mothers mated at PND70 | | | | |
|---|---|---|---|---|
| Treatment group | N | Gestation length (days) | N | Gestation weight gain (grams) |
| 1 | 13 | 22.1 ± 0.6 | 14 | 124.8 ± 13.2 |
| 2 | 11 | 21.6 ± 0.5 | 11 | 121.7 ± 17.1 |
| 3 | 11 | 22 ± 0.0 | 10 | 119.52 ± 31.3 |
| 4 | 9 | 22 ± 0.5 | 9 | 119.8 ± 16.7 |
| 5 | 9 | 21.8 ± 0.3 | 9 | 108.1 ± 30.7 |
| 6 | 10 | 21.5 ± 1.1 | 10 | 126.6 ± 9.6 |
| 7 | 11 | 21.8 ± 0.3 | 11 | 126.1 ± 21.3 |
| 8 | 13 | 21.6 ± 0.7 | 13 | 127.3 ± 14.4 |
| 9 | 11 | 22.3 ± 0.5 | 11 | 110.1 ± 29.5 |
| 10 | 13 | **22.2 ± 0.6**[2] | 11 | 121.8 ± 10.7 |
| 11 | 10 | **21 ± 0.9**[1] | 9 | 107.7 ± 17.8 |
| 12 | 9 | 18.4 ± 7.9 | 8 | 129.1 ± 15.6 |
| 13 | 9 | 20.7 ± 2.0 | 8 | 126.9 ± 20.9 |

[1]Significantly different from sham surgery controls ($p \leq 0.05$)
[2]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

Table 83: F2 generation litter size, percentage of males per litter, and pup survival for litters born to mothers mated at PND70

| Parental treatment group | Number of litters | Litter size (PND1) | 95% CI | Percentage males | 95% CI | Percentage of pups surviving PND1-4 | 95% CI | Percentage of pups surviving PND5-20 | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 14 | 12.8 | 2.5 | 54.7 | 8.0 | 96.8 | 3.5 | 99.0 | 2.0 |
| 2 | 13 | 14.5 | 1.2 | 48.1 | 10.8 | 91.4 | 16.7 | 89.4 | 16.6 |
| 3 | 13 | 14.4 | 1.4 | 52.8 | 4.8 | 98.1 | 2.4 | 99.0 | 2.3 |
| 4 | 11 | 12.9 | 3.3 | 50.1 | 9.6 | 100.0 | 0.0 | 91.3 | 10.4 |
| 5 | 11 | 13.7 | 3.2 | 46.9 | 7.9 | 87.9 | 19.8 | 98.8 | 2.8 |
| 6 | 11 | 13.1 | 1.7 | 51.1 | 9.0 | 81.8 | 19.8 | 91.7 | 9.8 |
| 7 | 12 | 13.7 | 1.9 | 54.7 | 8.3 | 89.2 | 18.2 | 95.5 | 10.1 |
| 8 | 15 | 14.3 | 2.1 | 47.4 | 8.4 | 92.4 | 14.3 | 98.2 | 3.9 |
| 9 | 12 | 12.7 | 2.5 | 48.2 | 6.8 | 87.2 | 19.1 | 98.9 | 2.5 |
| 10 | 17 | 13.6 | 0.8 | 53.6 | 6.9 | 94.1 | 12.5 | 96.8 | 3.9 |
| 11 | 14 | 11.7 | 2.1 | 52.0 | 10.9 | 96.5 | 5.8 | 98.7 | 2.8 |
| 12 | 10 | 13.0 | 2.0 | 47.6 | 7.4 | 96.9 | 5.7 | 100.0 | 0.0 |
| 13 | 12 | 13.6 | 1.7 | 49.4 | 11.8 | 98.8 | 1.9 | 97.6 | 3.6 |

Table 84: F2 generation average litter size and pup weight at PND 1 and PND 4

| Parental treatment group | Litters evaluated | Average Litter Size PND1 | 95% CI | Average Pup Weight PND 1 (grams) | 95% CI | Average Litter Size PND4 | 95% CI | Average Pup Weight PND 4 (grams) | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 14 | 12.8 | 2.5 | 7.3 | 0.9 | 12.4 | 2.5 | 10.5 | 1.4 |
| **2** | 13 | 14.5 | 1.2 | 6.6 | 0.6 | 13.2 | 2.6 | 10.2 | 0.9 |
| **3** | 13 | 14.4 | 1.4 | 6.8 | 0.4 | 14.4 | 1.0 | 9.7 | 0.9 |
| **4** | 11 | 12.9 | 3.3 | 6.8 | 1.2 | 12.5 | 3.6 | 10.6 | 2.1 |
| **5** | 11 | 13.7 | 3.2 | 6.7 | 0.4 | 13.1 | 3.4 | 9.8 | 1.2 |
| **6** | 11 | 13.1 | 1.7 | 6.4 | 0.7 | 11.2 | 3.0 | 9.8 | 1.8 |
| **7** | 12 | 13.7 | 1.9 | 6.8 | 0.9 | 12.1 | 3.1 | 10.1 | 0.7 |
| **8** | 15 | 14.3 | 2.1 | 6.7 | 0.5 | 13.1 | 2.9 | 10.1 | 0.8 |
| **9** | 12 | 12.7 | 2.5 | 7.2 | 0.8 | 11.8 | 2.9 | 10.5 | 1.7 |
| **10** | 17 | 13.6 | 0.8 | 6.8 | 0.7 | 12.9 | 1.9 | 9.7 | 0.8 |
| **11** | 14 | 11.7 | 2.1 | 7.0 | 0.8 | 11.2 | 2.4 | 10.9 | 1.5 |
| **12** | 10 | 13.0 | 2.0 | 6.6 | 0.7 | 12.6 | 2.1 | 9.4 | 1.2 |
| **13** | 12 | 13.6 | 1.7 | 6.8 | 0.6 | 13.4 | 1.7 | 9.6 | 1.3 |

Table 85: F2 average pup weight gain from PND5 though PND20

| Parental treatment group | Number of male pups used in weight gain calculation (PND5-20) | Male Pup Weight Gain PND 5-20 (grams) | 95% CI | Number of female pups used in weight gain calculation (PND5-20) | Female Pup Weight Gain PND 5-20 (grams) | 95% CI |
|---|---|---|---|---|---|---|
| 1 | 13 | 38.6 | 5.5 | 13 | 38.9 | 3.4 |
| 2 | 12 | 38.6 | 3.9 | 12 | 38.6 | 5.5 |
| 3 | 12 | 40.1 | 3.3 | 12 | 41.8 | 2.5 |
| 4 | 10 | 39.6 | 3.2 | 10 | 43.4 | 4.1 |
| 5 | 10 | 42.1 | 3.3 | 10 | 36.9 | 4.1 |
| 6 | 12 | 40.1 | 3.6 | 11 | 37.3 | 4.9 |
| 7 | 10 | 38.8 | 5.8 | 10 | 38.3 | 3.6 |
| 8 | 12 | 41.1 | 3.6 | 12 | 36.4 | 2.4 |
| 9 | 11 | 40.1 | 3.2 | 11 | 36.2 | 4.6 |
| 10 | 15 | 39.2 | 2.9 | 15 | 38.4 | 3.9 |
| 11 | 13 | 42.5 | 3.3 | 14 | 38.6 | 2.4 |
| 12 | 7 | 36.9 | 4.7 | 7 | 36.3 | 3.8 |
| 13 | 10 | 37.6 | 2.9 | 9 | 38.6 | 2.7 |

| Table 86: F2 male pup weights at PND 20, 60, and 90 | | | | | | |
|---|---|---|---|---|---|---|
| Treatment group | N | PND20 | N | PND60 | N | PND90 |
| 1 | 13 | 51.5 ± 6.4 | 15 | 365.7 ± 26.6 | 9 | 486.6 ± 80.2 |
| 2 | 12 | 50.7 ± 4.6 | 13 | 367.0 ± 41.4 | 5 | 520.1 ± 52.5 |
| 3 | 12 | 52.2 ± 3.7 | 10 | 400.7 ± 34.3 | 6 | 541.1 ± 62.9 |
| 4 | 10 | 52.4 ± 5.3 | 13 | 367.1 ± 44.9 | 7 | 515.2 ± 65.4 |
| 5 | 10 | 54.0 ± 3.9 | 16 | 354.4 ± 35.4 | 6 | 527.3 ± 64.1 |
| 6 | 12 | 51.0 ± 4.8 | 15 | 322.9 ± 71.7 | 8 | 457.1 ± 120.5 |
| 7 | 10 | 51.4 ± 6.1 | 7 | 323.7 ± 108.0 | 5 | 426.3 ± 165.4 |
| 8 | 12 | 53.2 ± 4.3 | 14 | 360.4 ± 47.2 | 6 | 565.1 ± 56.4 |
| 9 | 11 | 52.9 ± 4.6 | 14 | 385.2 ± 50.3 | 2 | 470.3 ± 781.4 |
| 10 | 15 | 51.3 ± 3.8 | 25 | 357.1 ± 27.1 | 16 | 502.0 ± 44.6 |
| 11 | 13 | 56.1 ± 4.4 | 23 | 395.4 ± 16.5 | 18 | 550.6 ± 27.8 |
| 12 | 7 | 48.2 ± 5.9 | 14 | 368.0 ± 34.8 | 11 | 552.2 ± 46.9 |
| 13 | 10 | 48.9 ± 4.4 | 19 | 361.9 ± 23.6 | 15 | 509.5 ± 50.3 |

| Table 87: F2 male pup weight gain PND 20-60 and 20-90 | | | | |
|---|---|---|---|---|
| Treatment group | N | PND 20-60 | N | PND 20-90 |
| 1 | 15 | 315.0 ± 23.3 | 9 | 433.9 ± 78.3 |
| 2 | 13 | 317.3 ± 40.7 | 5 | 468.8 ± 53.8 |
| 3 | 9 | 357.1 ± 35.0 | 6 | 488.8 ± 63.1 |
| 4 | 13 | 315.7 ± 44.8 | 7 | 461.0 ± 67.0 |
| 5 | 16 | 301.3 ± 35.5 | 6 | 475.4 ± 63.7 |
| 6 | 15 | 273.5 ± 69.1 | 8 | 411.6 ± 116.2 |
| 7 | 7 | 267.1 ± 110.6 | 4 | 357.8 ± 247.4 |
| 8 | 14 | 308.1 ± 46.2 | 6 | 512.0 ± 57.6 |
| 9 | 14 | 330.0 ± 50.4 | 2 | 412.7 ± 796.2 |
| 10 | 24 | 305.6 ± 27.4 | 15 | 453.7 ± 46.0 |
| 11 | 23 | 339.7 ± 16.0 | 18 | 494.1 ± 26.9 |
| 12 | 14 | 318.5 ± 32.5 | 11 | 501.9 ± 44.5 |
| 13 | 17 | 311.0 ± 24.0 | 14 | 457.9 ± 53.2 |

| Table 88: F2 female pup weights at PND 20, 60, and 90 | | | | | | |
|---|---|---|---|---|---|---|
| Treatment group | N | PND20 | N | PND60 | N | PND90 |
| 1 | 13 | 49.1 ± 6.1 | 17 | 231.7 ± 13.6 | 10 | 301.2 ± 35.8 |
| 2 | 12 | 51.2 ± 3.7 | 12 | 238.7 ± 16.0 | 5 | 296.0 ± 26.5 |
| 3 | 12 | 48.3 ± 3.5 | 13 | 245.3 ± 15.5 | 6 | 301.6 ± 38.3 |
| 4 | 10 | 50.4 ± 6.5 | 13 | 226.9 ± 12.2 | 8 | 295.3 ± 21.2 |
| 5 | 10 | 52.4 ± 2.6 | 13 | 228.8 ± 14.2 | 6 | 298.4 ± 27.2 |
| 6 | 11 | 49.8 ± 4.0 | 13 | 286.1 ± 47.6 | 5 | 343.9 ± 116.4 |
| 7 | 10 | 49.4 ± 5.9 | 8 | 231.5 ± 59.3 | 9 | 306.5 ± 78.6 |
| 8 | 12 | 51.2 ± 3.6 | 14 | 241.1 ± 24.9 | 4 | 354.1 ± 102.3 |
| 9 | 11 | 50.2 ± 4.5 | 15 | 231.4 ± 13.9 | 3 | 291.7 ± 78.3 |
| 10 | 15 | 49.0 ± 3.3 | 28 | 250.2 ± 22.1 | 15 | 290.5 ± 23.2 |
| 11 | 14 | 53.6 ± 4.1 | 27 | 252.5 ± 15.3 | 22 | 332.6 ± 27.8 |
| 12 | 7 | 45.4 ± 6.1 | 12 | 251.9 ± 22.5 | 12 | 319.0 ± 26.7 |
| 13 | 9 | 48.1 ± 5.6 | 18 | 239.9 ± 20.7 | 13 | 299.5 ± 24.0 |

| Table 89: F2 female pup weight gains at PND 20-60 and 20-90 | | | | |
|---|---|---|---|---|
| Treatment group | N | PND 20-60 | N | PND 20-90 |
| 1 | 17 | 181.8 ± 10.5 | 10 | 250.1 ± 32.7 |
| 2 | 12 | 187.8 ± 14.5 | 5 | 244.6 ± 23.3 |
| 3 | 11 | 201.0 ± 18.8 | 6 | 253.1 ± 43.1 |
| 4 | 13 | 175.8 ± 12.1 | 8 | 242.2 ± 21.4 |
| 5 | 13 | 176.8 ± 14.0 | 6 | 247.7 ± 27.4 |
| 6 | 13 | 236.1 ± 46.5 | 5 | 296.5 ± 112.8 |
| 7 | 8 | 181.2 ± 62.1 | 8 | 261.0 ± 90.1 |
| 8 | 14 | 191.6 ± 24.9 | 4 | 304.1 ± 104.3 |
| 9 | 15 | 179.1 ± 14.0 | 3 | 237.4 ± 75.7 |
| 10 | 27 | 201.8 ± 21.8 | 14 | 245.6 ± 22.4 |
| 11 | 27 | 198.9 ± 15.7 | 22 | 278.8 ± 27.8 |
| 12 | 12 | 205.0 ± 19.1 | 12 | 272.3 ± 23.3 |
| 13 | 16 | 192.7 ± 22.5 | 12 | 250.9 ± 23.5 |

Table 90: Hematology values measured in adult F2 rats following necropsy at 200 days post-implantation (± 95% Confidence Interval). n=(x).

| Endpoint | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WBC ($10^3$ µl) | 12.4 ± 3 (30) | 13.0 ± 2.8 (33) | 9.2 ± 1.6 (22) | 11.0 ± 2.4 (28) | 9.9 ± 2.3 (20) | 11.8 ± 2 (25) | 12.2 ± 1.1 (18) | 10.4 ± 1.1 (31) | 11.1 ± 1.5 (30) | 8.9 ± 1.6 (22) | 10.5 ± 1.9 (34) |
| % Lymphocytes | 85.8 ± 3.7 (29) | 88.2 ± 1 (32) | 86.9 ± 2.1 (24) | 86.2 ± 2.8 (27) | 86.0 ± 4 (20) | 86.9 ± 1.1 (23) | 87.8 ± 1.3 (18) | 88.1 ± 1.1 (31) | 88.3 ± 1.7 (30) | 89.6 ± 1.5 (23) | 89.8 ± 1.4 (33) |
| % Monocytes | 6.2 ± .7 (28) | 7. ± .5 (32) | 6.5 ± .8 (24) | **7.5 ± .8 (27)[1]** | 6.3 ± 1 (18) | **7.6 ± .8 (23)[1]** | 6.6 ± .7 (18) | 6.6 ± .6 (31) | 6.3 ± .5 (30) | 6.4 ± .8 (23) | 5.9 ± .6 (33) |
| % Granulocytes | 6.6 ± 2.2 (28) | 4.8 ± .8 (32) | 6.6 ± 1.7 (24) | 5.3 ± 1.3 (27) | 6.5 ± 2.2 (20) | 5.4 ± .8 (23) | 5.6 ± 1.3 (18) | 5.4 ± .8 (31) | 5.41 ± 1.4 (30) | 4 ± 1 (23) | 4.3 ± 1.0 (33) |
| # Lymphocytes | 11.4 ± 2.1 (28) | 10.3 ± .9 (32) | 8.0 ± 1.44 (22) | 8.8 ± 1.4 (27) | 8.5 ± 1.7 (20) | 11.1 ± 1.3 (23) | 10.7 ± 1.1 (18) | 9.1 ± .9 (31) | 9.7 ± 1.2 (30) | 8.3 ± 1.2 (21) | 9.5 ± 1.5 (33) |
| # Monocytes | .8 ± .2 (28) | .8 ± .1 (32) | .6 ± .1 (22) | .7 ± .15 (27) | .7 ± .2 (18) | 1 ± .2 (23) | .8 ± .1 (18) | .7 ± .1 (31) | .7 ± .1 (30) | .6 ± .1 (20) | .7 ± .1 (32) |
| # Granulocytes | 1.1 ± .8 (28) | .6 ± .1 (32) | .6 ± .1 (21) | .6 ± .2 (27) | .8 ± .6 (19) | .7 ± .1 (23) | .7 ± .1 (18) | .6 ± .1 (31) | .7 ± .4 (30) | .4 ± .2 (20) | .6 ± .3 (31) |
| RBC ($10^6$ µl) | 7 ± .9 (25) | 7.6 ± .2 (16) | 7 ± 1.4 (11) | 7.4 ± .3 (24) | 6.7 ± 1 (17) | 6.7 ± 1.2 (18) | 7.5 ± .2 (15) | 7.1 ± .6 (25) | 7.1 ± .7 (20) | 6.9 ± 1 (19) | 6.3 ± 1.2 (22) |
| HgB (g/dl) | 14.5 ± 1.3 (33) | 15.5 ± .3 (34) | 14.8 ± 1.0 (28) | 15.1 ± .3 (30) | 14.2 ± 1.5 (22) | 14.3 ± 1.5 (31) | 15.1 ± .2 (20) | 14.3 ± .9 (32) | 14.8 ± .9 (30) | 14.1 ± 1.3 (34) | 14.1 ± 1.3 (35) |
| Hematocrit % | 41.4 ± 5.1 (25) | 43.1 ± 1.4 (16) | 40.1 ± 7.9 (11) | 43 ± 1.9 (23) | 39.5 ± 6.2 (17) | 40.3 ± 7.2 (18) | 43.4 ± 1.2 (15) | 40.2 ± 3.5 (25) | 41.4 ± 4.1 (20) | 39.1 ± 6 (19) | **36.9 ± 5.9 (21)[1]** |
| MCV $Mm^3$ | 58.2 ± .9 (33) | 57.1 ± .8 (33) | 57.6 ± 1.8 (27) | 60.5 ± 6.4 (29) | 58 ± 1.1 (22) | 58.3 ± 1.0 (31) | 57.3 ± 1.1 (20) | 56.5 ± 1.0 (32) | 56.7 ± 1.3 (30) | 57.2 ± 2 (33) | **62.1 ± 7.68 (28)[1]** |
| MCH (pg) | 20.7 ± .8 (25) | 19.7 ± .3 (16) | 19.4 ± .7 (11) | 20.1 ± .5 (24) | 20.9 ± 1.2 (17) | 21.1 ± 1.3 (18) | 19.8 ± .3 (15) | 20.2 ± 1 (25) | 19.9 ± .3 (20) | 19.5 ± .8 (19) | 20.1 ± .4 (21) |
| MCHC | 34.4 ± 2.6 | 33.3 ± | 34.1 ± 1.9 | 34.8 ± | 35.9 ± 2.7 | 35.8 ± 3 | 34.4 ± | 35.7 ± | 34.4 ± | **34.2 ± 1.4** | 35.8 ± .8 |

Table 90: Hematology values measured in adult F2 rats following necropsy at 200 days post-implantation (± 95% Confidence Interval). n=(x).

| Endpoint | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (g/dl) | (26) | 2.8 (17) | (11) | 1.1 (23) | (17) | (18) | .8 (15) | 2.0 (25) | .8 (20) | **(19)**[2] | (21) |
| RDW% | 12.7 ± .4 (33) | 12.6 ± .5 (33) | 14.3 ± 2.2 (28) | 14 ± 2.1 (29) | 12.5 ± .7 (22) | 12.5 ± .5 (31) | 12.5 ± .5 (20) | 12.7 ± .4 (32) | 12.8 ± .5 (30) | 14.7 ± 2.6 (33) | 17.2 ± 4.2 (31) |
| PLT ($10^3$ µl) | 935.1±103.6 (32) | 994.7 ± 62.1 (33) | 1036.7±118. (27) | 923.8 ± 80.5 (28) | 927.3±114.0 (22) | 983.5±125.6 (31) | 1010.9 ± 66. (20) | 1040 ±142.2 (32) | 937.3 ± 99.8 (30) | 895.9±128.8 (33) | 898.2±150.2 (27) |
| MPV (fl) | 7.5 ± .4 (32) | 7.0 ± .2 (33) | 7.4 ± .4 (27) | 7.6 ± .3 (28) | 7.3 ± .4 (22) | 7 ± .3 (31) | 7.1 ± .3 (20) | 7.0 ± .2 (32) | 7.18 ± .3 (30) | 7.8 ± .3 (33) | 7.5 ± .3 (26) |

[1]Significantly ($p \leq 0.05$) different from Group 1 (sham surgery controls)
[2] Significantly ($p \leq 0.05$) different from Group 11 (inert implant controls)

Table 91: Sperm motion parameters for F2 pups measured following euthanasia and necropsy on PND200

| Parental treatment group | F2 pups evaluated | % motile | % progressive | VAP[a] (μm/sec) | VSL[b] (μm/sec) | VCL[c] (μm/sec) | ALH[d] (μm) |
|---|---|---|---|---|---|---|---|
| 1 | 12 | 182.5 ± 12.5 | 127.9 ± 9.8 | 344.4 ± 21.3 | 15.4 ± 0.8 | 80.5 ± 4.2 | 24.6 ± 3.6 |
| 2 | 11 | 200.7 ± 13.4 | 139.1 ± 8.7 | 384.8 ± 25.1 | 16.5 ± 0.8 | **83.3 ± 3.6[1]** | 25.3 ± 2.1 |
| 3 | 13 | 175.5 ± 11.7 | 121.7 ± 8.4 | 337.9 ± 26.4 | 15.4 ± 0.9 | 81.7 ± 5.6 | 24.5 ± 2.8 |
| 4 | 10 | 186.1 ± 18.4 | 129.0 ± 13.8 | 350.2 ± 28.8 | 15.4 ± 1.0 | 79.5 ± 5.1 | 24.6 ± 4.1 |
| 5 | 10 | 178.3 ± 11.3 | 122.6 ± 8.6 | 341.9 ± 22.7 | 15.2 ± 1.3 | 82.4 ± 3.6 | 23.7 ± 2.6 |
| 6 | 13 | 179.2 ± 13.4 | 124.8 ± 9.2 | 336.0 ± 16.9 | 14.7 ± 0.9 | 73.0 ± 12.2 | 23.2 ± 6.0 |
| 7 | 9 | 165.4 ± 11.7 | 114.6 ± 8.0 | 321.2 ± 25.1 | 14.8 ± 1.0 | 78.4 ± 9.9 | 22.3 ± 4.3 |
| 8 | 13 | 176.8 ± 10.0 | 123.1 ± 7.2 | 347.9 ± 24.4 | 15.2 ± 1.2 | 78.7 ± 8.9 | 23.9 ± 3.9 |
| 9 | 11 | 169.3 ± 11.6 | 116.0 ± 8.8 | 322.5 ± 24.2 | 14.2 ± 1.2 | 80.7 ± 4.0 | 21.6 ± 3.3 |
| 10 | 17 | 174.5 ± 14.2 | **121.5 ± 10.0[2]** | 335.8 ± 22.2 | 14.8 ± 1.1 | 81.9 ± 3.7 | 25.5 ± 5.0 |
| 11 | 21 | 170.2 ± 7.6 | 113.5 ± 6.6 | 326.8 ± 18.0 | 14.0 ± 0.9 | 79.6 ± 3.3 | 19.9 ± 2.9 |

[a]VAP= Average path velocity; [b]VSL= Progressive straight line velocity; [c]VCL= Tract speed velocity;
[d]ALH= Lateral sperm head displacement
[1]Significantly ($p \leq 0.05$) different from Group 1 (sham surgery controls)
[2]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

Table 92: Average caudal sperm concentrations for F2 pups measured following euthanasia and necropsy on PND200

| Parental treatment group | F2 pups evaluated | # of Cells | Concentration ($10^6$ cells/mL) | Concentration ($10^6$ cells/gram tissue) |
|---|---|---|---|---|
| 1 | 12 | 194.5 ± 56.5 | 22.5 ± 6.5 | 1056.8 ± 333.6 |
| 2 | 12 | 160.6 ± 26.2 | 18.6 ± 3.0 | 874.5 ± 201.1 |
| 3 | 14 | 152.2 ± 21.0 | 17.6 ± 2.4 | 763.5 ± 126.1 |
| 4 | 13 | 186.1 ± 45.3 | 21.5 ± 5.2 | 945.8 ± 262.7 |
| 5 | 9 | 120.6 ± 46.5 | 14.0 ± 5.4 | 607.5 ± 244.2 |
| 6 | 17 | 220.6 ± 43.4 | 25.5 ± 5.0 | 1121.8 ± 241.6 |
| 7 | 13 | 174.7 ± 43.0 | 20.2 ± 5.0 | 940.7 ± 199.0 |
| 8 | 14 | 209.4 ± 31.4 | 24.2 ± 3.6 | 1075.9 ± 182.7 |
| 9 | 11 | 150.1 ± 45.9 | 17.4 ± 5.3 | 858.9 ± 312.1 |
| 10 | 18 | 157.2 ± 19.6 | 18.2 ± 2.2 | 879.2 ± 107.2 |
| 11 | 2 | 160.5 ± 146.1 | 18.6 ± 17.2 | 958.8 ± 1363.3 |
| 12 | 4 | 215.3 ± 215.1 | 24.9 ± 24.8 | 1204.0 ± 1113.4 |
| 13 | 20 | 180.5 ± 34.7 | 20.9 ± 4.0 | 1022.3 ± 173.4 |

Table 93: Mean absolute weights (± 95% confidence interval) in grams of major organs and organ systems of male F2 adult rats that underwent necropsy on PND90. n=(x).

| Parental treatment group | Sex organs | Liver | Kidneys | Heart | Spleen | Brain |
|---|---|---|---|---|---|---|
| 1 | 8.7 ± 1.1 (12) | 22.8 ± 1.9 (14) | 4.5 ± 0.4 (14) | 2.0 ± 0.2 (14) | 0.9 ± 0.2 (13) | 1.9 ± 0.1 (13) |
| 2 | 8.4 ± 0.8 (9) | 21.5 ± 3.7 (10) | 4.4 ± 0.6 (10) | 2.1 ± 0.3 (10) | 0.9 ± 0.2 (9) | 2.0 ± 0.2 (10) |
| 3 | 8.1 ± 0.7 (8) | 21.8 ± 2.6 (11) | 4.1 ± 0.3 (11) | 2.3 ± 0.2 (11) | 0.9 ± 0.1 (11) | 2.0 ± 0.1 (9) |
| 4 | 8.5 ± 1.4 (9) | 20.6 ± 2.8 (10) | 4.3 ± 0.6 (10) | 2.1 ± 0.3 (10) | 0.8 ± 0.2 (11) | 2.0 ± 0.2 (11) |
| 5 | 8.2 ± 1.1 (10) | 22.6 ± 2.3 (9) | 4.4 ± 0.4 (10) | 2.2 ± 0.2 (10) | 1.0 ± 0.2 (9) | 2.1 ± 0.1 (10) |
| 6 | 8.7 ± 1.1 (12) | 22.9 ± 2.9 (12) | 4.4 ± 0.5 (11) | **2.4 ± 0.2[1] (12)** | 1.0 ± 0.1 (11) | 2.1 ± 0.2 (13) |
| 7 | 8.1 ± 0.6 (11) | 20.9 ± 1.9 (11) | 4.2 ± 0.4 (11) | 2.2 ± 0.2 (11) | 1.0 ± 0.1 (11) | 2.1 ± 0.1 (11) |
| 8 | 8.7 ± 0.5 (13) | 21.3 ± 2.8 (12) | 4.2 ± 0.3 (13) | 2.1 ± 0.2 (13) | 1.1 ± 0.2 (12) | 2.1 ± 0.1 (13) |
| 9 | 8.3 ± 0.5 (9) | 21.0 ± 2.0 (11) | 4.1 ± 0.4 (10) | 2.2 ± 0.2 (11) | 0.9 ± 0.1 (11) | 2.1 ± 0.2 (9) |
| 10 | 9.4 ± 0.6 (13) | 22.8 ± 2.2 (15) | 4.0 ± 0.4 (16) | 1.9 ± 0.2 (17) | 0.8 ± 0.1 (17) | 1.9 ± 0.1 (17) |
| 11 | 9.9 ± 1.0 (10) | 23.3 ± 2.3 (13) | 3.9 ± 0.4 (13) | 2.1 ± 0.2 (14) | 0.7 ± 0.1 (14) | 1.9 ± 0.1 (14) |

[1]Significantly ($p \leq 0.05$) different from Group 1 (sham surgery controls)

Table 94: Mean relative weights (± 95% confidence interval) in grams of major organs and organ systems of male F2 adult rats that underwent necropsy on PND90. n=(x). All values x $10^{-3}$.

| Parental treatment group | Sex organs | Liver | Kidneys | Heart | Spleen | Brain |
|---|---|---|---|---|---|---|
| 1 | 18.6 ± 3.8 (6) | 45.5 ± 7.3 (8) | 8.5 ± 2.0 (8) | 4.0 ± 0.8 (8) | 1.8 ± 0.7 (7) | 4.1 ± 0.9 (7) |
| 2 | 16.1 ± 2.4 (3) | 43.2 ± 11.3 (4) | 7.6 ± 0.7 (4) | 4.2 ± 1.3 (4) | 1.5 ± 0.7 (4) | 3.8 ± 1.1 (4) |
| 3 | 15.0 ± 2.4 (4) | 43.8 ± 5.2 (5) | 7.5 ± 0.4 (5) | 4.3 ±0.9 (5) | 1.8 ± 0.3 (5) | 3.9 ± 0.6 (5) |
| 4 | 18.9 ± 5.6 (4) | 43.4 ± 2.3 (5) | 8.6 ± 0.6 (5) | 4.1 ± 0.3 (5) | 1.8 ± 0.7 (5) | 4.1 ± 0.6 (5) |
| 5 | 14.2 ± 5.8 (4) | 45.3 ± 12.9 (3) | 8.1 ± 0.5 (4) | 4.3 ± 1.1 (4) | 2.0 ± 0.6 (4) | 3.8 ± 0.5 (4) |
| 6 | 16.7 ± 5.9 (6) | 44.9 ± 11.3 (6) | 9.4 ± 3.7 (6) | 5.8 ± 1.2 (5) | 2.0 ± 0.6 (5) | 4.4 ± 1.2 (6) |
| 7 | 19.3 ± 7.8 (4) | 52.7 ± 28.4 (4) | 10.4 ± 6.6 (4) | 5.1 ± 3.2 (4) | 2.6 ± 1.7 (4) | 4.8 ± 3.4 (4) |
| 8 | 16.0 ± 1.9 (6) | 40.2 ± 14.9 (5) | 7.3 ± 1.2 (6) | 3.9 ± 0.5 (6) | 1.9 ± 0.3 (5) | 3.8 ± 0.4 (6) |
| 9 | 17.7 ± 24.3 (2) | 48.4 ± 42.8 (2) | 8.9 ± 14.7 (2) | 4.6 ± 8.9 (2) | 2.1 ± 3.1 (2) | 3.9 ± 2.0 (2) |
| 10 | 20.4 ± 3.2 (10) | 45.9 ± 3.1 (12) | 8.5 ± 0.9 (12) | **3.7 ± 0.3**[1] **(13)** | 1.7 ± 0.4 (13) | 4.0 ± 0.5 (13) |
| 11 | 19.2 ± 1.7 (8) | 41.1 ± 3.4 (10) | 6.8 ± 0.5 (10) | 4.0 ± 0.5 (11) | 1.4 ± 0.3 (11) | 3.6 ± 0.3 (11) |

Table 95: Mean absolute weights (± 95% confidence interval) in grams of major organs and organ systems of female F2 adult rats that underwent necropsy on PND90. n=(x).

| Parental treatment group | Sex organs | Liver | Kidneys | Heart | Spleen | Brain |
|---|---|---|---|---|---|---|
| 1 | 0.8 ± 0.1 (13) | 12.5 ± 1.0 (14) | 2.5 ± 0.2 (14) | 1.4 ± 0.2 (14) | 0.7 ± 0.1 (14) | 1.6 ± 0.1 (14) |
| 2 | 1.1 ± 0.3 (8) | 11.8 ± 1.5 (8) | 2.3 ± 0.5 (9) | 1.7 ± 0.4 (8) | 0.8 ± 0.4 (9) | 1.7 ± 0.2 (9) |
| 3 | 1.0 ± 0.2 (12) | 11.9 ± 0.8 (12) | 2.7 ± 0.2 (12) | 1.6 ± 0.1 (12) | 0.8 ± 0.2 (12) | 1.9 ± 0.2 (12) |
| 4 | 1.0 ± 0.3 (10) | 11.9 ± 1.2 (10) | 2.6 ± 0.4 (10) | 1.5 ± 0.2 (10) | 0.7 ± 0.2 (10) | 1.8 ± 0.2 (10) |
| 5 | 1.2 ± 0.2 (9) | 12.8 ± 1.4 (9) | 2.7 ± 0.3 (9) | 1.7 ± 0.2 (7) | 0.8 ± 0.3 (9) | 1.8 ± 0.2 (9) |
| 6 | 1.0 ± 0.1 (10) | 11.9 ± 1.0 (10) | 2.5 ± 0.4 (10) | 1.7 ± 0.3 (10) | 0.8 ± 0.2 (10) | 1.8 ± 0.2 (10) |
| 7 | 0.9 ± 0.2 (10) | 12.1 ± 1.4 (12) | 2.7 ± 0.4 (12) | 1.6 ± 0.3 (12) | 0.8 ± 0.1 (12) | 1.6 ± 0.2 (12) |
| 8 | 1.1 ± 0.2 (11) | 11.6 ± 1.1 (11) | 3.1 ± 1.1 (11) | **1.9 ± 0.3[1] (10)** | 0.7 ± 0.1 (11) | 1.7 ± 0.2 (11) |
| 9 | 1.2 ± 0.1 (11) | 11.7 ± 0.8 (11) | 2.9 ± 0.4 (11) | 1.8 ± 0.4 (8) | 0.8 ± 0.2 (11) | 2.0 ± 0.2 (11) |
| 10 | 1.0 ± 0.2 (16) | 11.9 ± 1.0 (16) | 2.5 ± 0.2 (16) | 1.6 ± 0.2 (16) | 0.5 ± 0.1 (16) | 1.7 ± 0.1 (16) |
| 11 | 1.1 ± 0.3 (11) | 12.4 ± 1.1 (12) | 2.5 ± 0.3 (13) | 1.8 ± 0.3 (13) | 0.6 ± 0.2 (12) | 1.7 ± 0.2 (13) |

[1]Significantly ($p \leq 0.05$) different from Group 1 (sham surgery controls)

Table 96: Mean relative weights (± 95% confidence interval) in grams of major organs and organ systems of female F2 adult rats that underwent necropsy on PND90. n=(x). All values x $10^{-3}$.

| Parental treatment group | Sex organs | Liver | Kidneys | Heart | Spleen | Brain |
|---|---|---|---|---|---|---|
| 1 | 2.5 ± 0.7 (9) | 38.0 ± 3.1 (8) | 8.8 ± 1.3 (9) | 4.6 ± 1.1 (9) | 1.9 ± 0.4 (9) | 5.3 ± 0.5 (9) |
| 2 | 3.5 ± 2.3 (2) | 37.1 ± 5.7 (2) | 8.3.± 4.4 (2) | 5.0 ± 0.2 (2) | 2.0 ± 4.1 (2) | 5.1 ± 3.7 (3) |
| 3 | 3.1 ± 1.4 (5) | 42.9 ± 5.9 (5) | 9.7 ± 1.4 (5) | 5.6 ± 0.9 (5) | 2.6 ± 0.9 (5) | 6.5 ± 0.9 (5) |
| 4 | 1.3 ± 3.0 (6) | 43.5 ± 2.8 (6) | 9.6 ± 1.2 (6) | 5.7 ± 1.2 (6) | 2.5 ± 1.0 (6) | 6.4 ± 1.0 (6) |
| 5 | 3.8 ± 0.9 (4) | 43.0 ± 7.1 (4) | 9.0 ± 0.9 (4) | 3.8 ± 0.9 (2) | 2.1 ± 0.6 (4) | 5.6 ± 0.8 (4) |
| 6 | 2.6 ± 1.3 (4) | 39.9 ± 13.8 (3) | 7.9 ± 3.9 (4) | 5.1 ± 1.2 (4) | 2.2 ± 1.0 (4) | 5.3 ± 1.8 (4) |
| 7 | 2.9 ± 0.9 (6) | 44.4 ± 4.9 (6) | 9.8 ± 1.2 (7) | 5.6 ± 1.3 (7) | 3.0 ± 0.8 (7) | 5.6 ± 1.0 (7) |
| 8 | 3.4 ± 1.9 (4) | 33.5 ±11.0 (4) | 7.4 ± 2.2 (3) | 5.0 ± 0.9 (3) | 1.7 ± 1.1 (4) | 4.8 ± 2.2 (4) |
| 9 | 4.2 ± 3.1 (3) | 41.0 ± 8.9 (3) | 13.2 ± 38.5 (2) | 6.2 ±NA (1) | 2.6 ± 1.3 (3) | 6.2 ± 2.2 (3) |
| 10 | 3.6 ± 0.7 (10) | 40.8 ± 2.1 (10) | **9.0 ± 1.1[1] (10)** | 5.4 ± 0.5 (10) | 1.8 ± 0.6 (10) | **6.0 ± 0.5[1] (10)** |
| 11 | 3.5 ± 0.9 (10) | 37.9 ± 2.2 (11) | 7.6 ± 0.9 (12) | 5.4 ± 0.9 (12) | 2.0 ± 0.5 (11) | 5.1 ± 0.6 (12) |

[1]Significantly different ($p \leq 0.05$) from Group 11 (inert implant) controls

Reportable Outcomes (1 June 2003 – 31 May 2005):

- Three manuscripts were written describing the study results and were accepted for publication. The manuscripts are:

  Arfsten, D.P., Schaeffer, D., Johnson, E.W., Cunningham, R.J., Still, K.R., Wilfong, E.R. 2005. Effect of implanted depleted uranium (DU) on male reproductive success, sperm concentration and sperm velocity. Environ. Res. *Accepted for publication.*

  Arfsten DP, Bekkedal M, Rossi J III, Grasman KA, Thitoff AR, Healey LB, Rutkiewicz JM, Jung AE, Johnson EW, Lohrke SR, Wilfong ER, Schaeffer DJ, Still KR. 2005. Effect of depleted uranium (DU) implanted in adult rats for 30 days on reproductive success, P1 maternal care, and the development and survival of F1 offspring. J. Toxicol. Environ. Health Part A. 68:1-31.

  Arfsten, D.P., Still, K.R., Ritchie, G.D. 2001. A review of the effects of uranium and depleted uranium (DU) exposure on reproduction and fetal development. Toxicol Industrial Health 17:180-191 (Written and accepted for publication in 2003).

- Five posters describing the study results were presented at various scientific meetings over the funding period:

  Arfsten, D.P., E.E. Wilfong, E.W. Johnson, D.J. Schaeffer, K.R. Still. 2005. Effect of implanted depleted uranium (DU) on male rat reproductive success, sperm concentration and motion Abstract 545. Presented at the 44th Annual Meeting of the Society of Toxicology, March 6-10, 2005, New Orleans, LA.

  Healey, LB, Rutkiewicz JM, Lohrke SR, Arfsten DP, Grasman KA. 2005. T-cell mediated immunity in adult Sprague-Dawley rats implanted with depleted uranium. Presented at the 44th Annual Meeting of the Society of Toxicology, March 6-10, 2005, New Orleans, LA.

  Thitoff, A.R., Jung, A.E., Johnson, E.W., Lohrke, S.L., Stutler, S.A., Ketzenberger, B., Still, K.R., Arfsten, D.P. 2004. Multi-generation reproductive toxicity study of implanted depleted uranium in rats: results from the 1st generation. The Toxicologist. 78: #1034. 43rd Annual Meeting of the Society of Toxicology, March 21-25, 2004, Baltimore, Maryland.

  Rossi, J. III, Bekkedal, M.Y.V., McInturf, S.M., McDougle, F.J., Lenger, A., Allen, C.T., and Arfsten, D.P. 2004. Failure to detect differences in neurobehavioral development of rats following *in utero* and pre-weaning exposure to depleted uranium. 21st International Neurotoxicology Conference. Infant and Child Neurotoxicity Studies: Subtle and Long-Term Effects. Focusing on Methylmercury, PCBs, Heptachlor and Other Persistent Pollutants. February 10-14, 2004. Ala Moana Hotel, Honolulu, Hawaii.

Arfsten, D.P. and Still, K.R. 2002. Potential mechanisms for the toxic effects of Depleted Uranium (DU) alloy on rat reproduction and fetal development. Theories and Practices in Toxicology and Risk Assessment Conference, April 15 – 18, 2002. Cincinnati, OH.

- Three talks describing the study results were presented during the funding period:

  Arfsten DP, Jederberg WW, Still KR. 2004. Multi-generation reproductive toxicity study of implanted depleted uranium in rats: Results from the 1st generation. Presented at the 2004 USACHPPM 7th Annual Force Health Protection Conference, 9-12 August, 2004, Albuquerque, N.M.

  Arfsten DP, Jederberg WW, Still KR. 2004. Multi-generation reproductive toxicity of depleted uranium in rats. Presented at the American Industrial Hygiene Conference & Expo 2004, May 8-13, 2004, Atlanta, GA.

  Arfsten, D.P., Jederberg, W.W., and Still, K.R. 2004. Results of a large-scale study of the potential reproductive effects of depleted uranium fragments in rodents. Presented at the 2004 Toxicology and Risk Assessment Conference, April 26-30, Cincinnati, OH.

Conclusions:

*P1 generation*

- Implanted DU pellets solubilzed in the SD rat as evidenced by increasing levels of urinary uranium with increasing number of implanted pellets when evaluated at 27 and 114 days after implantation surgery. Chemical analysis of all major tissues found that uranium tended to accumulate in the teeth, femur, and kidney in rats implanted with DU pellets for 200 days.

- Macrophages surrounding implanted DU pellets contained black material similar in composition to the implanted DU pellets suggesting macrophages were involved in transport of some uranium from the implantation site

- Implantation of up to 20, 1 x 2 mm DU pellets in the gastrocnemius of rats did not have a negative impact on their health or well being when studied for up to 200 days after implantation:

    - Average body weight and body weight gains of animals implanted with DU were not different than those of negative control animals

    - Serum chemistry test results of DU-implanted adult rats were negative for markers of liver and kidney toxicity

    - Foreign body reactions were common findings for DU implantation sites. No evidence of carcinogenesis or tissue proliferation was observed when the implantation sites were evaluated histopathologically. Implantation site carcinogenesis would not be expected because of the relatively short time between implantation and necropsy (200 days).

    - Histopathological analysis of major organs, including the male and female reproductive tract, did not find evidence of toxicity or tissue alterations associated with DU implantation

    - For animals implanted with 20 DU pellets, hematology values for percent monocytes and platelet concentration were significantly different than for animals implanted with 20 Ta steel pellets; however, all values for each parameter were within 20% of the mean sham surgery control values suggesting the finding was not likely of biological significance

    - The sperm motility parameter VCL was significantly higher for animals implanted with 20 DU or 20 Ta steel pellets as compared with negative controls, therefore, the effect dose not appear to be related to implanted DU

    - Neurobehavioral and immune function tests did not produce evidence of a negative impact of DU implantation on these endpoints

*F1 generations*

- There were no findings to suggest that implantation of up to 20, 1 x 2 mm DU pellets in the gastrocnemius of rats had a negative impact on adult reproductive success or offspring litter size, litter weight, survival and development
    - Mating success, gestation length and gestation weight gain were similar for DU-implanted animals as compared with controls when mated at 30 and 120 days post-implantation
    - There was no evidence from maternal retrieval testing that maternal care was impacted negatively as a result of DU implantation
    - There was no evidence of negative effects of DU implantation on litter size and weight, pup survival, sex ratio, weight gain, and early development.
    - Gross observation of PND4 and observation and detailed necropsy of PND20 pups did not find evidence of increased frequency for developmental abnormalities among offspring from DU-implanted animals
    - Adolescent and adult stage weight gain and survival of offspring from DU-implanted animals were similar to those of control animals
    - Weights for several major organs were compared between DU and control pups at PND200; no evidence was found for an effect of parental DU implantation on tissue weight and development
    - Histopathological analysis of major organs, including the male and female reproductive tract, did not find evidence of toxicity or tissue alterations associated with parental DU implantation
    - Sperm motility and concentrations were compared between DU and control pups at PND90 and PND200; no evidence was found for an effect of parental DU implantation on tissue weight and development
    - Mean WBC concentrations for Group 10 pups were found to be significantly higher than for controls when assessed at PND90 and PND200; however, all values for each parameter were within 20% of the mean sham surgery control values suggesting the finding was not likely of biological significance

- Mean MCV was identified as being significantly different from sham surgery controls for F1a Groups 3, 4, 8, 9, and 10; however, all values for each parameter were within 20% of the mean sham surgery control values and are within normal range for MCV for the SD rat (53-59 $mm^3$)

- Neurobehavioral and immune function tests did not produce evidence of a negative impact of DU implantation on F1 neurobehavior or immune function

*F2 generation*

- The mating success rate for F1b adults to produce the F2 generation was significantly lower than for P1 matings (69% versus 86% and 91%, respectively), but success was not related to DU implantation levels in P1 parents with higher dose pups (Group 10-13) having significantly higher success rates than sham surgery controls (Group 1) and Groups 2-9. This suggests that the lower overall mating success rate may have been due to environmental factors. Mating produced at least 10 litters for each treatment Group.

- Overall, there were no findings to suggest that pups derived from DU-implanted parents 2 generations removed were affected by parental DU implantation

  - Gestation length and gestation weight gain of F1b mothers were similar among the 13 treatment groups

  - Litter size and weight, pup survival, sex ratio, weight gain, and early development were similar among the 13 treatment groups

  - Gross observation of PND4 and observation and detailed necropsy of PND20 pups did not find evidence of increased frequency for developmental abnormalities among the 13 treatment groups

  - Adolescent and adult stage weight gain and survival of offspring through PND90 were similar among the 13 treatment groups

  - Weights for several major organs were compared between the 13 treatment groups at PND90; no evidence was found to suggest that tissue weight and development was different for treatment Groups 2-13 as compared with pups from Group 1

  - Necropsy and gross observation of major organs, including the male and female reproductive tract, did not find evidence for any abnormalities among all F2 pups

  - F2 sperm motility and concentrations were similar among the 13 study groups when compared at PND90

- Differences in % monocytes, % hematocrit, MCV, and MCHC were identified by Dunnett's multiple comparisons test; however, all values for each parameter were within 20% of the mean sham surgery control values suggesting the finding was not likely of biological significance

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